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Science

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Science

COVER The cellular changes of lightly pigmented *golden* zebrafish show a striking resemblance to those of lighter skinned humans. The zebrafish pigment gene *slc24a5* is functionally conserved across evolution; a single base change in its human ortholog may play a role in pigment variation in human populations. See page 1782. [Image: J. Mest and J. Cheng]

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Number 5755

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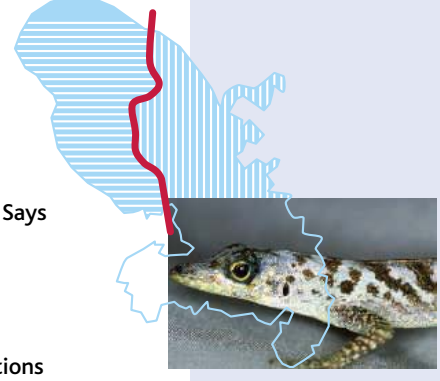

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Why is he so attracted?

Roberto Gradnik
Regional Vice-President, Serono

Italy is currently a key strategic location for Serono, thanks to its competitive costs, excellent research centres and high productivity, comparable to Switzerland or anywhere in Europe. This is why Serono chose to strengthen its Italian presence with a new R&D centre in Rome in 2004. Italian Life Sciences industry is the third largest in Europe, a world market leader in the sector attracting major global companies. The presence of high performing research centres with a proven track record of achievements in Healthcare research and a strong synergy between academia and industry has led to the creation of specialized biotechnology clusters, with excellent perspectives in Oncology and Neurosciences. An array of recent applications in the Biomedical, Bioinformatics, Biomechanics and Nano-biotechnology fields is catching foreign investors' attention.
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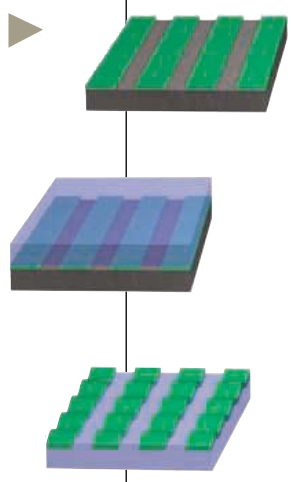
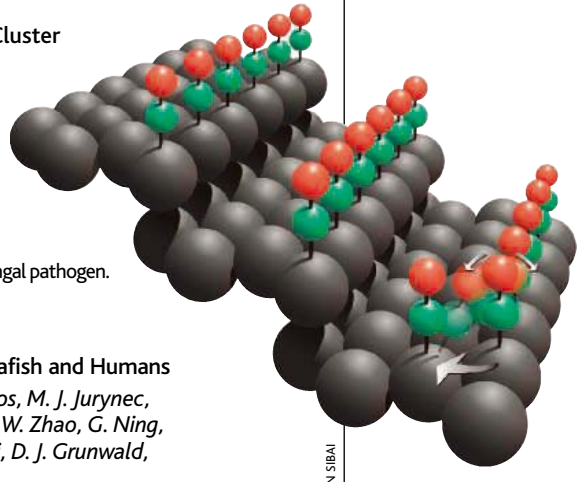


TECHNICAL COMMENT ABSTRACTS



CREDITS: YASMIN SIBAI

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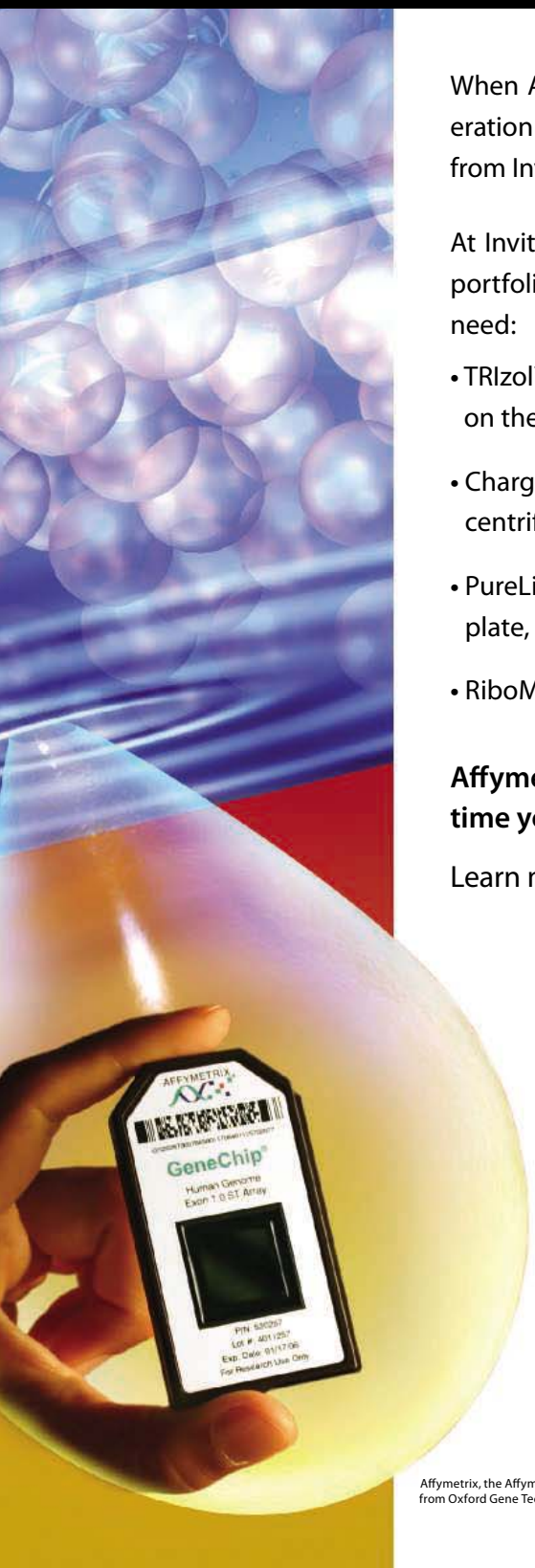
Opt for innovation



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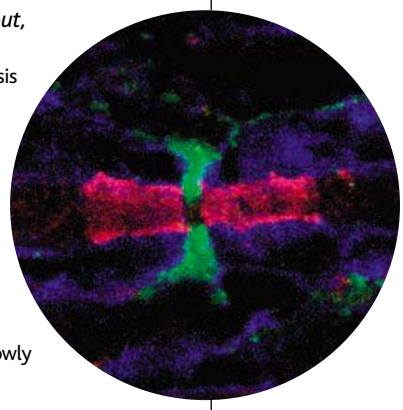

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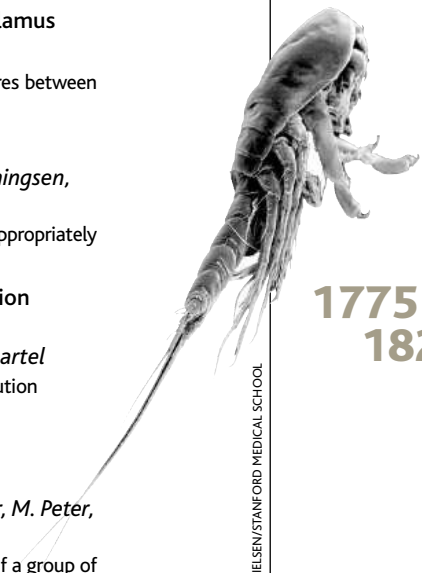
1813



CREDITS: ALEX NIELSEN/STANFORD MEDICAL SCHOOL

1775 & 1824

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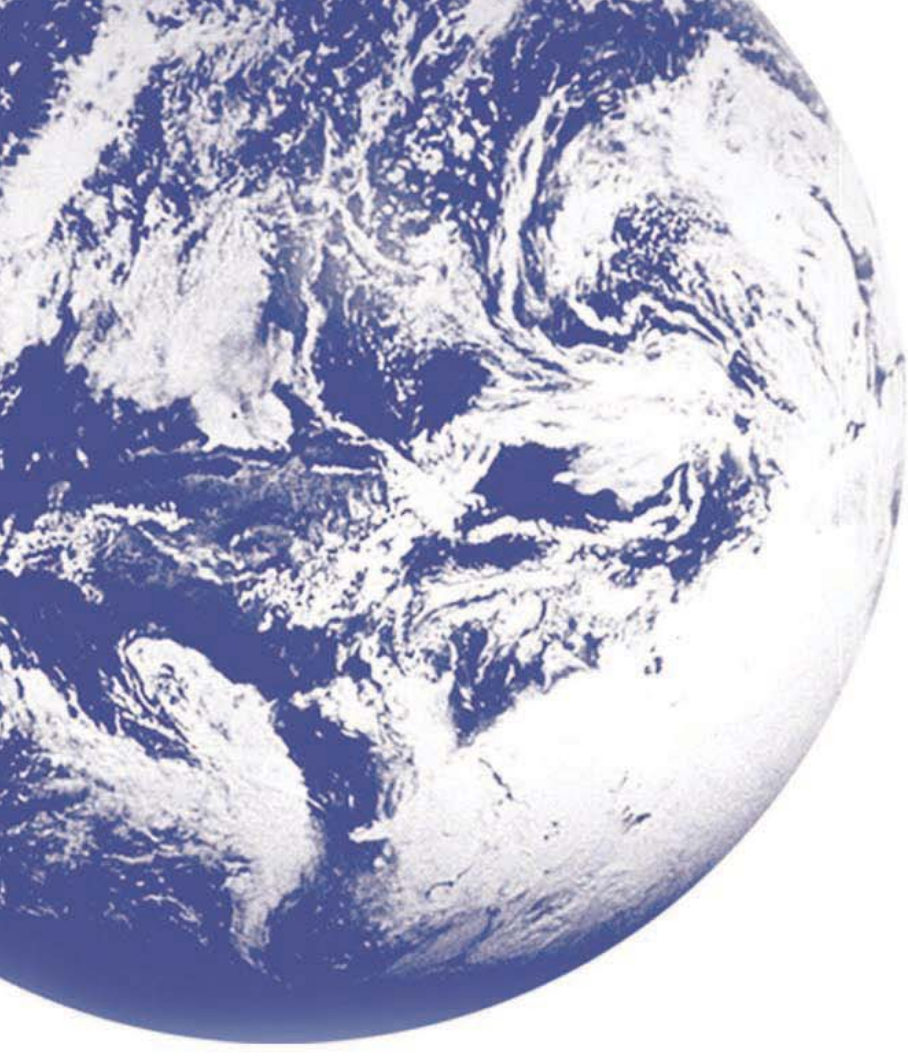
Contents continued ▶



The Defense Threat Reduction Agency, Joint Science and Technology Office is proud to announce the opening of the 2007 physical program call for research proposals. This competitive program is open to private, academic, government, and international scientists. The closing date for whitepapers is Jan. 23, 2006. Topic areas include:

Detection

Defense chem/bio sensor technologies that respond faster and to a broader scope of threats at lower levels. Technologies are sought to increase standoff range and decrease false alarms.

Protection

Reduce the physiological and logistical burdens associated with individual and collective protection against WMD threats through reduced weight, reduced power consumption, new filter technologies, and improved shelter systems.

Decontamination

Develop lighter-weight and self-decontaminating materials that protect against traditional and non-traditional threats. This includes active and passive surface coatings, sorbents, and reactive solutions to decontaminate various equipment surfaces.

Modeling & Simulation/Battlespace

Integrate information from sensors with atmospheric modeling to provide timely CBRN hazard information for advance warning and battlespace management.

Threat Agent Science

Conduct research to gain a better understanding of threat agent signatures, field behavior, and effects to enable approaches to new technologies.

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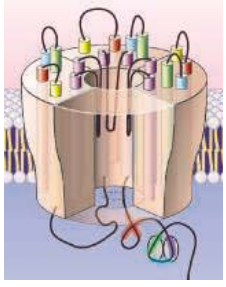
Radically different aging?



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Review: Regulation of Voltage-Gated Ca^{2+} Channels by Calmodulin *D. B. Halling, P. Aracena-Parks, S. L. Hamilton*
Calmodulin's versatility allows it to mediate both inhibition and facilitation of voltage-gated calcium channel function.

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Dr. Paul Verkade, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany
Dr. Verkade works with the Leica EM PACT2 & RTS High Pressure Freezer.

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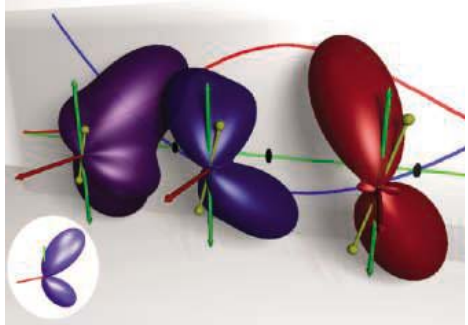
Begin with a Backflip

The initiation of diffusion of molecules on surfaces is mainly thought of in terms of translational motion. **Backus *et al.*** (p. 1790, published online 10 November; see the Perspective by **Ueba and Wolf**) followed the diffusion of CO molecules on a stepped Pt surface with ultrafast vibrational spectroscopy by using changes in CO stretching frequencies to distinguish different adsorption sites. Photoexcitation of the CO with a laser pulse revealed very fast motion (a time constant of only 500 femtoseconds) that was associated with CO rotation rather than translation. Density functional theory calculations show that the excitation of frustrated rotational motion of the CO molecule is needed for the molecule to hop to an adjoining adsorption site.

The Reptile-Dinosaur-Bird Conundrum

Examination of the histology of fossil bones has shown that most dinosaurs, like birds and mammals today, attained their adult size at about the same age after a period of rapid growth, independently of environmental factors. In contrast, many reptiles adjust their growth in response to temperature and other factors, and may attain adult size at rather different ages. By examining a large collection of fossils from central Europe, **Sander and Klein** (p. 1800; see the news story by **Gramling**) now show that the most common Triassic dinosaur, the large prosauropod *Plateosaurus engelhardti* grew more like turtles, snakes, and alligators, unlike later dinosaurs, whose growth response resembles that of birds and mammals.

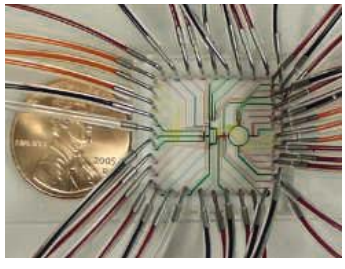


Comparing Correlations

Quantum mechanics offers an exact solution to the forces binding an electron to a proton in the hydrogen atom. However, adding just one more proton and electron to the system presents an intractable complication arising from the correlated motion of the electrons. **Vanroose *et al.*** (p. 1787) have improved the approximate solution by numerical computation. They analyze the trajectories of both electrons upon double ionization of the hydrogen molecule by a single photon, specifically focusing on the influence of changing the internuclear separation. The result is distinct from the path taken on double ionization of the helium atom, These findings indicate that significant correlation effects stem from a molecular geometry (an electron pair shared between two protons), as opposed to an atomic geometry (an electron pair symmetrically surrounding two protons).

Small Reactors

Positron emission tomography (PET) achieves local sensitivity in medical imaging of organs by detecting the emissive decay of isotopically unstable molecular probes. This instability also requires the rapid and efficient synthesis of probe compounds. **Lee *et al.*** (p. 1793) have built a computer-controlled device, roughly the size of a penny, for optimizing the speed and cost of such preparations. The micrometer-scale valves and channels achieve rapid mixing and solvent exchange, and efficient heat transfer, as demonstrated in the multistep synthesis of ^{18}F-radiolabeled 2-deoxy-2-fluoro-D-glucose, the most widely used PET probe.



The Genetics of Skin Pigmentation

Little is known about the specific genes that contribute to the variations in human skin color. An exciting clue has now emerged from an unlikely source, a tiny aquarium fish. Working with a mutant line of zebrafish called golden, whose stripes are paler than those in wild-type fish, **Lamason *et al.*** (p. 1782; see the cover and the news story by **Balter**) found that the altered pigmentation was caused by a mutation in the *slc24A5* gene, which encodes a protein potentially involved in cation exchange. The gene is highly conserved in vertebrates, and expression of the human gene in the golden zebrafish restored wild-type pigmentation. European populations carry a slightly different version of the *slc24A5* gene than do African and East Asian populations. A genetic polymorphism that changes one amino acid in the coding region of the gene correlates with skin pigmentation levels, which suggests that *slc24A5* may contribute to skin color in humans.

Hiding in the Long Grass

Since the seminal work of MacArthur and Wilson on the theory of island biogeography, studies on this topic have focused mainly on the relation of species richness with island parameters such as area, distance, and habitat variability. The population biology of individual species in the island context has received much less attention. **Schoener *et al.*** (p. 1807; see the Perspective by **Thorpe**) report results from an experiment using *Anolis* lizards and an introduced lizard predator on small islands in the Bahamas archipelago. In the absence of the predator, there was a highly regular (decreasing) correlation of lizard survival to a key habitat variable (vegetation height). In the presence of the predator, the situation was nearly reversed, such that prey survival was highest in the tallest vegetation.

Modification of Electrical Synapses

The brain has two main types of synapses, chemical and electrical. Electrical synapses represent a major form of communication between interneurons in the mammalian nervous system. They play an important role in synchronization of activity in local cell populations because their speed and reliability allows signals to spread across whole networks at a time scale that is sufficient to preserve precise timing of signals between distant neurons. In spite of these potentially vital functions, electrical synapses have generally been regarded as stereotypic and nonflexible. However, **Landisman and Connors** (p. 1809) found that transmission across electrical synapses

CONTINUED ON PAGE 1739

CREDITS (TOP TO BOTTOM): VANROOSE *ET AL.*; LEE *ET AL.*

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can undergo long-term modifications just like chemical synapses. The modulation depends on activation of metabotropic glutamate receptors, which presumably trigger intracellular signal cascades modulating the connexins that constitute the electrical synapses.

Snails on the Rampage

There has been unprecedented and massive die-off of southeastern United States salt marshes during the past 5 years, with potentially serious consequences for coastal protection and integrity. **Silliman *et al.*** (p. 1803) surveyed more than 1200 kilometers of coastline and found high-density fronts of plant-grazing snails (~1500 individuals per square meter) mowing down marsh plants at 11 of 12 die-off sites. Die-off was initiated by drought-induced stress. Snail fronts developed at the edges of the die-off zones, and then spread across remaining healthy areas. These interactions between climatic and trophic factors may lead to further degradation or even collapse of these ecologically and economically important systems.

Inhibiting Brain Repair

Neuronal axons in the mammalian central and peripheral nervous system are generally ensheathed in myelin that is generated by nonneuronal cells. In response to injury in the peripheral nervous system, new axons can sprout from unmyelinated gaps called the Nodes of Ranvier, but this response rarely occurs in the central nervous system (CNS). **Huang *et al.*** (p. 1813, published online 17 November) have identified a precursor oligodendrocyte cell type whose processes envelope nodes in the CNS and inhibit axon sprouting. The processes express a glycoprotein previously thought exclusive to compact myelin. Mice lacking the glycoprotein exhibited abnormal node formation and nodal axon sprouting. Overcoming the inhibitory nature of these cells may be clinically important in recovery from injury.

MicroRNA Management of the Genome

MicroRNAs (miRNAs), small, ~22-nucleotide noncoding RNAs that have been found in most of the plants and animals so far studied, generally regulate gene expression by suppressing the activity of messenger RNAs (mRNA) bearing complementary target sequences. These targets, or "seeds," are apparently only seven to eight nucleotides long, and so, all things being equal, should occur randomly throughout the genome with relatively high frequency. **Farh *et al.*** (p. 1817, published online 24 November) now show that all things are not equal: Expression of regulated seed-bearing mRNAs correlates with the presence of the appropriate miRNA. However, nonregulated mRNAs present at high levels in miRNA-expressing tissues have a paucity of complementary seed matches in their sequence. Thus, miRNAs are influencing the expression, the evolution, or both of the majority of mRNAs.

Just-in-Time Competency

Many bacteria can take up exogenous DNA, an ability known as natural competence. The causative agent of cholera, *Vibrio cholerae*, is not known to have this property, but somehow it has clearly acquired virulence attributes, including cholera toxin, from some other source. *V. cholerae* does possess the genes used by other bacteria to assemble the necessary machinery for DNA uptake, for example, type IV pili. **Meibom *et al.*** (p. 1824; see the Perspective by **Bartlett and Azam**) now show that a chitin (which can be found in the exoskeleton of crabs, a natural host for the bacteria) triggers *V. cholerae* to produce pili, and to release and exchange functional DNA. This competency remained unnoticed in a pathogen that has been studied for 60 years, which suggests that other noncompetent bacteria may become so under the appropriate growth conditions.

CREDIT: SILLIMAN *ET AL.*

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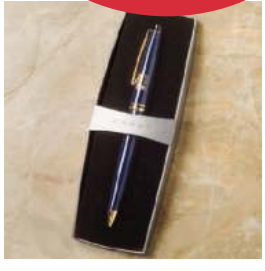

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Doing More for Kate

When Kate graduated from Lincoln High School, she had a budding interest in science. Taking college-level advanced placement courses in biology and chemistry during her senior year had been challenging, but a combination of enthusiastic teachers and supportive classmates brought her unanticipated satisfaction—she was learning how nature worked and had made a good start at analyzing it as a scientist would.

It took only 1 year of science classes at a large research university to turn Kate into a business major. Her general chemistry textbook was similar in content to the one she'd used in high school. But the class was so enormous that she only knew the professor as a speck in the distance. The laboratory section was taught by a teaching assistant who was struggling to learn English, but that didn't matter much because the acid/base titration was the same experiment that Kate had done in high school. Moreover, the pressure to memorize equations and work on assigned problems dampened Kate's enthusiasm for grappling with the underlying concepts.

So why should a research scientist reading this account care about what happened to Kate? After all, hasn't it always been this way? There is a laissez-faire attitude among some that although university science classes are tough, those who are really "cut out for it" will survive to populate the next generation of scientists. But we should care, and there are two reasons why.

First, the pipeline issue; illustrated here with reference to the United States, but a problem in many other countries as well. The number of Ph.D. degrees in science and engineering granted by U.S. universities increased by 45% from 1974 to 2004, somewhat more than the 37% growth in the country's population. But the doctoral degrees granted to U.S. citizens increased by only 11%, making non–U.S. citizens, most holding temporary visas, largely responsible for our keeping pace with the country's need for scientists. Clearly, something is turning Kate and her classmates away from careers in science.

Second, the future of the world is at stake! That's not melodrama. Never have exciting new developments in science been more tightly connected to real dilemmas in public policy. If the electorate distrusts science and doesn't understand how scientists explore and interrogate the natural world, how will they vote on issues ranging from stem cell research and global climate change to the teaching of intelligent design in our schools? In addition to full-time scientists, we need educated citizens who can think critically about the science and technology choices so prominent in contemporary political life.

Science and Howard Hughes Medical Institute (HHMI) are committed, each in their own ways, to revitalizing science education. Therefore, we are pleased to collaborate and bring the readers of *Science* innovative educational ideas in each month of 2006.* We want to showcase new approaches to teaching that work even in large lecture classes, or bring other disciplines, such as physics and computer sciences, together with biology into a single course. Learning is not a spectator sport, and through active involvement in the material, students will understand and retain concepts much better. We want to explore how to connect research and teaching for the benefit of both student and professor. We want to help faculty do what they would all love to do: teach better with less struggle. Above all, we hope to increase general interest in, and knowledge about, science; no matter what path our students embark on.

Why *Science*? Because it's widely read by scientists around the world, many of whom share a primary commitment to research and a conviction that the successor generation of scientists must be nurtured. If they agree with us that science and the teaching of science are inseparable, they are an audience we must reach.

We researchers pride ourselves on thinking scientifically in our laboratories. We gather data, formulate hypotheses, and suspect our own conclusions enough to test them rigorously. And we always want to apply the best technology available to our problems. When scientists step out of the lab into the classroom, they can apply these same principles: finding out what their students already know, reworking their methods to enhance understanding, and applying technology to support those efforts. This scientific approach to teaching science is what we will highlight in the upcoming issues of *Science*.

Thomas Cech
Donald Kennedy

Thomas Cech is president of HHMI in Chevy Chase, Maryland. Donald Kennedy is Editor-in-Chief of *Science*.

Science and HHMI will work with an advisory committee on the essay series. *Science* will have full editorial responsibility for the content. Essays may be submitted for consideration through our Web site at www.submit2science.org.

10.1126/science.1123580

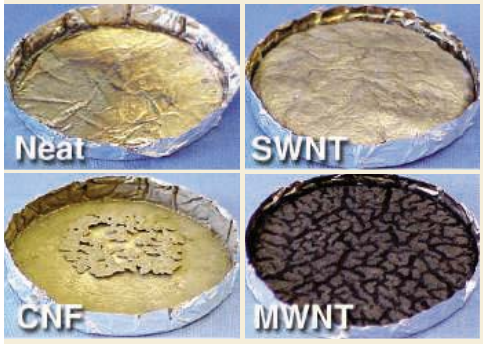
EDITORS' CHOICE

edited by Gilbert Chin

MATERIALS SCIENCE

Nanotube Firefighters

When polymers are heated and reach the temperature at which they begin to decompose, bubbles often form beneath the surface because the boiling points of the degradation products are usually lower than the decomposition temperature of the parent polymer. The evolution of these bubbles prevents the formation of a solid layer of char, which would insulate the rest of the polymer from further heating. With the advent of restrictions on halogenated flame-retardant additives, nanoscale reinforcing materials, such as clay particles, have been investigated as alternatives.



The residue of poly(methyl methacrylate) with various nanoadditives after heating.

Kashiwagi *et al.* have found that carbon nanotubes and nanoparticles can also act both as reinforcing materials and as flame retardants, and in some cases can surpass the performance of nanoclay materials. Coaxing the asymmetric fibers into a continuous network structure is the key to reducing bubbling. At fixed loads under radiant heat, the best results were obtained using single-walled carbon nanotubes (SWNTs), which left a residue with an undulating surface but no deep cracks. In contrast, multiwalled carbon nanotubes (MWNTs) yielded only islands of protection, and neither carbon nanofibers (CNF) nor carbon black particles helped very much. Flame retardancy was found to correlate with rheology, because the best materials showed a gel-like response, which matches their ability to form networks. — MSL

Nat. Mater. **4**, 928 (2005).

CHEMISTRY

Building a Better Wacker

The Wacker oxidation is a well-established method for the conversion of olefins to aldehydes and ketones. The reaction involves activation of the olefin toward water addition by a palladium catalyst, followed by regeneration of the catalyst by oxygen. In general, however, the regeneration step cannot be accomplished directly, but instead requires a copper or quinone derivative to shuttle electrons between Pd and O_2.

Mitsudome *et al.* show that a judicious choice of solvent eliminates the need for the co-catalyst. Using $PdCl_2$ in dimethylacetamide (DMA) solvent, they achieve efficient conversion of long-chain (up to C_{20}) terminal olefins to the corresponding 2-ketones on treatment with water under O_2 pressure. The catalyst tolerates hydroxyl and cyano groups and can be recycled several times after heptane extraction. Electrochemical studies suggest that DMA lowers the oxidation potential of the catalyst in its Pd(0) state, thereby promoting direct oxidation by O_2. — JSY

Angew. Chem. Int. Ed. 10.1002/anie.200502886 (2005).

PSYCHOLOGY

Frozen in Time

Humans may be unique in being aware of their own mortality. In any case, being reminded that we are, in fact, mortal is apt to evoke feelings of anxiety and to call forth mechanisms for alleviating or managing our reactions to lives being extinguished. One such strategy is to seek reinforcement of one's worldview, which has the consequence of skewing our opinions of others (and others' actions) toward the extremes of good (in accord with one's views) and bad. Furthermore, these valuations may very well become fixed at their best or worst if the other person has died.

Eylon and Allison provide evidence for the immutability of judgments in the form of two experiments in which subjects were assessed for the change in their valuations when a good person (fictitious in the first case, real in the second) was described as having behaved immorally and, conversely, when a bad person was reported as having acted meritoriously. They found that the decrement in positive ratings and the increase in negative ratings were both smaller when the persons in question were dead versus still alive, suggesting that our impressions of people, favorable or not, become resistant to change when they die. — GJC

Candles in the wind.

Pers. Soc. Psychol. Bull. **12**, 1708 (2005).

IMMUNOLOGY

Awakening HIV

One of the pressing issues in HIV research is understanding the mechanisms of viral latency, in which small numbers of memory $CD4^+$ T cells harbor a transcriptionally silent form of the integrated provirus. Because this latent virus can be reactivated and because it exists in this dormant form within a long-lived population of lymphocytes, it represents a life-long reservoir.

To overcome the in vivo paucity of latently infected memory cells, Williams *et al.* studied a human T cell line containing a single integrated provirus and found that RNA polymerase II did not bind to the proviral long terminal repeat (LTR) because of alterations in the chromatin structure that had been induced by the binding of the histone deacetylase enzyme HDAC1 to the LTR. Inhibition of HDAC1 or knockdown of NF-κB p50 (which recruits and complexes with HDAC1) were sufficient for the production of short nonproductive viral transcripts, and full viral transcription could be achieved by coexpressing the viral transactivating protein Tat. Establishing this mechanism in primary $CD4^+$ T cells will be the next step in determining whether combinations of

CONTINUED ON PAGE 1745

CREDITS (TOP TO BOTTOM): KASHIWAGI *ET AL.*, *NAT. MATER.* 4, 928 (2005); ABBIE ROWE/NATIONAL PARK SERVICE/JOHN FITZGERALD KENNEDY LIBRARY; REUTERS; CIA

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HDAC1 inhibition and Tat activation will prove viable as a means of overcoming latency in the clinic. — SJS

EMBO J. 10.1038/sj.emboj.7600900 (2005).

GENETICS

Pressure Under Pressure

Hypertension is an extremely common disorder that, left untreated, can lead to stroke, heart disease, and kidney failure. Individuals of African descent are at greater risk of developing high blood pressure than are those of European descent, and this may reflect adaptations to distinct environmental selection pressures experienced by ancestral populations. For example, ancient human populations living in hot humid climates where salt was scarce would likely have a physiology adapted to maximize salt retention (which would concomitantly increase blood vessel tone), but this selective pressure would be lost once populations moved to cooler regions.

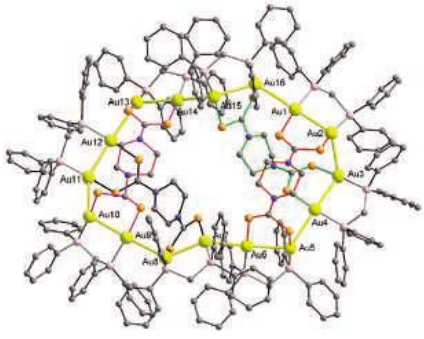
Young *et al.* present genetic data consistent with the hypothesis that differential susceptibility to hypertension among modern humans is due to climatic adaptation during the out-of-Africa expansion. Studying worldwide variation in five genes implicated in blood pressure regulation, they find that the prevalence of allelic variants that would increase heat adaptation (and hence hypertension susceptibility) is significantly greater in populations living at low latitudes or in hot wet climates than in those at high latitudes or cold dry climates. In addition, using data from an epidemiologic study of blood pressure in 52 different populations, they conclude that a major portion of the worldwide variation in blood pressure can be accounted for by latitude and a variant allele of GNB3, the beta–3 subunit of guanine nucleotide–binding protein. — PAK

PLoS Genet. 10.1371/journal.pgen.0010082.eor (2005).

CHEMISTRY

Chiral Golden Rings

The self-assembly of a large chiral aggregate with luminescent properties from achiral building blocks is reported by Yu *et al.*, who have exploited aurophilic interactions between Au(I) atoms to drive assembly. Two equivalents of the Au(I) dimer, $[Au_2(dppm)Cl_2]$ where dppm is the bridging bis(diphenylphosphino)methane ligand, bind to piperazine-1,4-dicarbodithiolate in anhydrous methanol; the thiol groups add a second bridging group to two gold dimers. This compound crystallizes as a tetramer in which the 16 gold atoms form a continuous loop: The two pairs of gold atoms from one monomer bind to the ends of a pair from an adjacent monomer, and two sets of bridging groups end up on each side of the loop. This interleaved cyclic assembly imparts chirality on the tetramer, which crystallizes with a 70% preference for one form in each sample prepared (but with essentially equal probability of either handedness for any given sample). The tetramer also displays intense green phosphorescence. — PDS



The crystal structure of the tetramer (Au, yellow; N, blue; P, pink; S, orange; C, gray).

J. Am. Chem. Soc. 10.1021/ja0565727.

HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

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Galanin Antagonists as Antidepressants

The neuropeptide galanin influences a broad range of processes in the central and peripheral nervous systems. Swanson *et al.* used two small molecules that selectively inhibit the Gal_3 receptor subtype to help define the effects mediated through this receptor in behavioral studies of anxiety and depression in three rodent model systems. They compared the effects of the inhibitors to those of chlordiazepoxide (a benzodiazepine anxiolytic) and fluoxetine (an antidepressant). In several assays, including the social interaction test and the forced swim test, the Gal_3 inhibitors showed acute and chronic antidepressant and anxiolytic effects equal to those of the control drugs, suggesting that Gal_3-selective agonists may be useful therapeutics. — LBR

Proc. Natl. Acad. Sci. U.S.A. **102**, 17489 (2005).

CREDITS: YU ET AL., J. AM. CHEM. SOC. 10.1021/JA0565727

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NetWatch

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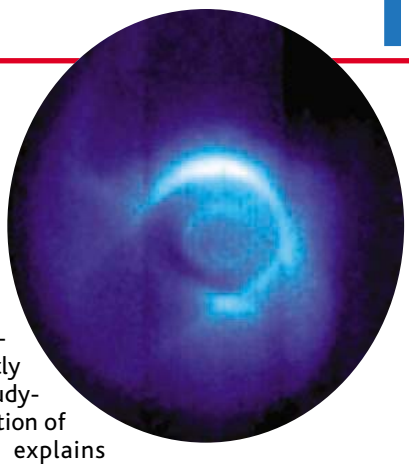
EDUCATION

Catch Some Rays



Cosmic rays spew from the sun, hurtle out of the remains of supernovas, and escape from other extraterrestrial sources. The speeding space particles, which constantly pelt Earth, interest astronomers studying questions such as the composition of the galaxy. NASA's Cosmicopia explains cosmic rays and related topics such as space weather for students and the public. Subjects include Earth's magnetosphere, the magnetic cloak around the planet that rebuffs many cosmic rays. The site also offers a Q&A written by experts, a timeline of ray research, and links to news stories. Above, a false-color image illuminates the magnetosphere.

helios.gsfc.nasa.gov

TOOLS

Species Crawl

iSpecies provides as much information as a good Internet encyclopedia, yet it doesn't contain a single Web page. The new search engine from systematist Roderic Page of the University of Glasgow in the U.K. furnishes what Web aficionados call a mashup. Instead of housing static pages about different species, it compiles a profile of your selected organism by linking to molecular, taxonomic, and other sites. Enter "aardvark," for example, and iSpecies tracks down images of the snouty mammal, protein and DNA sequences, a list of abstracts from recent papers, and a classification synopsis from the Integrated Taxonomic Information System.

www.ispecies.org

WEB LOGS

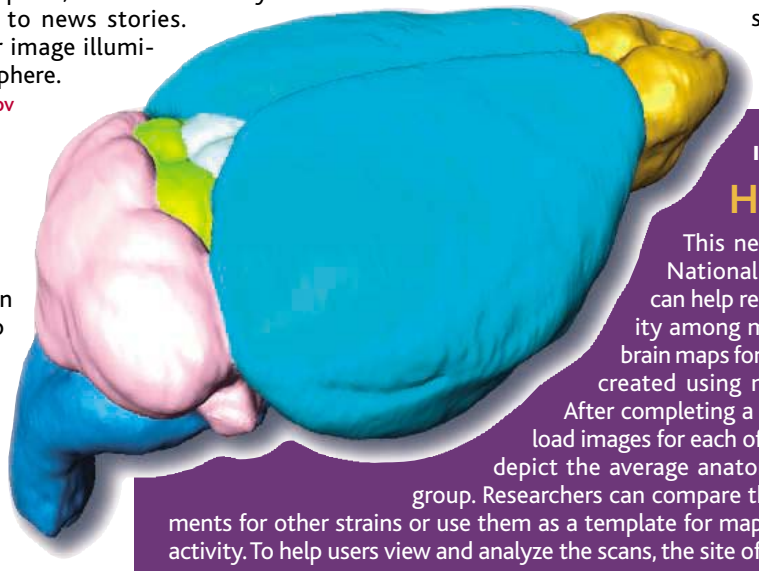
Bioethics Banter

With an egg-donation scandal at a top cloning lab, continued skirmishing over stem cells in the United States, and last month's first-ever face transplant, 2005 has given bioethicists plenty to contemplate. To follow the latest twists in these and other science stories with social impact, dive into the Web log launched in September 2004 by the editors of the *American Journal of Bioethics*. Although the journal's Web site offers some news, the blog format allows broader coverage and better explanations of issues, according to the three editors, who write most of the material. Its opinionated posts have highlighted developments such as classical musicians' use of beta blockers to quell stage fright and the current controversy over how South Korean stem cell pioneer Woo Suk Hwang's lab obtained human eggs (*Science*, 2 December, p. 1402). You'll also find newspaper commentaries co-written by a site editor.

blog.bioethics.net

IMAGES

Head Cases



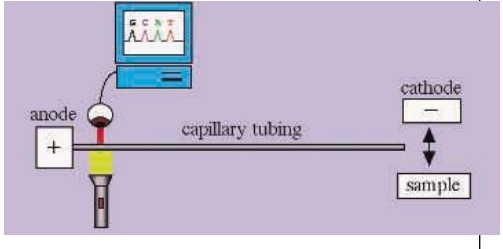
This new image bank from Brookhaven National Laboratory in Upton, New York, can help researchers studying neural variability among mouse strains. Stored here are 3D brain maps for the C57BL/6J strain, a lab favorite, created using magnetic resonance microscopy. After completing a free registration, users can download images for each of 10 rodents studied. Other atlases depict the average anatomy and the variation within the group. Researchers can compare the images to structural measurements for other strains or use them as a template for mapping data on gene and metabolic activity. To help users view and analyze the scans, the site offers free software.

www.bnl.gov/CTN/mouse

EDUCATION

Avoid Lab Mix-Ups

Nested or real-time PCR? Western, Southern, or Northern blotting? Newbies struggling to keep genomic methods straight can get help at this primer written by biologist Malcolm Campbell of Davidson College in North Carolina. Methods for Genomics isn't a lab manual but instead briefly explains more than 50 widely used techniques and pieces of equipment. With diagrams and animations, the site helps students grasp lab staples such as electrophoresis (right) and more advanced methods such as the Cre/*lox*P recombination system for deleting specific sections of DNA. The content ties in with a text Campbell uses in his classes, but it also works as a standalone resource.



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This Week

PAGE 1751
Dinosaurs' growth spurt

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Meeting of the minds on innovation

STEM CELLS

Korean University Will Investigate Cloning Paper

SEOUL AND TOKYO—Embattled Korean stem cell scientist Woo Suk Hwang and his university have bowed to pressure for an investigation into a growing list of questions about a landmark paper he and colleagues published in *Science* in June 2005 (17 June, p. 1777). On 12 December, Seoul National University (SNU), where Hwang works, announced it will conduct an investigation at the scientist's request. This follows a 7 December petition for an investigation from 30 SNU faculty members to university president Un Chan Chung. Prompted initially by anonymous allegations made on a public Web site about irregularities in the paper, scientists in Korea and elsewhere are calling for the paper's key DNA fingerprinting tests to be redone by an independent researcher.

Back to work. Cloning researcher Woo Suk Hwang returned to his lab on 12 December. He had been hospitalized for several days suffering from symptoms of stress and fatigue.

(As *Science* went to press, one of Hwang's co-authors, Gerald Schatten of the University of Pittsburgh in Pennsylvania, asked *Science* to remove his name from the paper.)

Meanwhile, stem cell researchers elsewhere are worried about the possible fallout. The lab's as-yet-unreplicated feat of creating human embryonic stem (ES) cell lines that match the DNA of patients inspired a global ramp-up in stem cell efforts. Such ES cell lines might one day provide replacement cells genetically matched to a patient suffering from Parkinson's disease or diabetes. Hwang's team not only showed that producing such ES cell lines was possible but also that it could be done efficiently, with relatively few donated oocytes per cell line. Alan Colman, head of Singapore-based ES Cell International and a member of the team that produced Dolly the sheep, the first cloned mammal, says, "I'd still like to believe this is a case of sloppy presentation but good science." If the results of the paper do not hold up, he says it could set the field back to a time when many thought the research "was too difficult and inefficient to pursue." It would also provide ammunition to opponents of the research, he says.

The latest revelations center on the DNA fingerprinting in the paper's supplementary online material first posted on 19 May 2005; the fingerprinting data purportedly show that the ES cells are genetically identical to the patients. There are also new allegations about another set of images in the online material that Hwang last week told editors at *Science* had been erroneously duplicated (*Science*, 9 December, p. 1595). All the scientific questions can apparently be traced to anonymous observations about the paper posted on an Internet message board hosted by the Biological Research Information Center (BRIC) (bric.postech.ac.kr). BRIC officials declined to comment, but a senior Korean scientist who has followed the postings agreed to discuss the issue provided he not be identified. (The Korean scientists contacted for this article requested anonymity because they fear a backlash against what are perceived to be attacks on Hwang, who has become a national icon. "This issue is now completely beyond the realm of science," one laments.)

The senior scientist says the message board writer, who claims to be a life science researcher, first pointed out the possibility of duplicated images early on 5 December Korea time. Hwang's e-mailed notice of problems with duplicate images arrived at *Science*'s editorial offices on 4 December at 11:29 p.m. Eastern Standard Time, which would have been 1:29 p.m. on 5 December in Korea, or several hours after the images were posted on the message board.

On 7 December, a critique of the DNA fingerprinting results appeared on the BRIC site. DNA fingerprinting shows a genetic match between two samples when peaks in the traces line up. But because the height and shapes of peaks are influenced by random factors, they should not be identical. The anonymous poster pointed out that the traces for several cell lines appear to be identical to the traces from the respective patients. In other cases, the background noise on the two traces looks very similar.

Alec Jeffreys, a genetic fingerprinting expert at the University of Leicester, U.K., said in an e-mail that "some of the traces do look unusually similar in peak shape and background noise." He declined to comment further without seeing the original data.

The anonymous poster also notes that Hwang's admission of duplicated images does not include other images that appear to have been duplicated.

The postings have elicited a flurry of responses. The consensus, says the senior scientist following the BRIC postings, seems to be that if Korean scientists don't take the lead in reviewing the paper, "the integrity of the Korean scientific community might be questioned by the world community."

Two of the 30 SNU professors who signed the petition asking for an investigation told *Science* the group first learned of the questions surrounding the paper from the BRIC discussion. One of the two professors contacted by *Science* says that they are not trying to discredit Hwang. "Dr. Hwang is a pioneer researcher in the field, and his studies should be pursued. We just see a serious need for a review."

The investigation comes amid a flurry of claims and counterclaims in the Korean media. On 10 December, a Korean news Web site called Pressian reported that it had seen a transcript from an unaired documentary by the Korean Munhwa Broadcasting Corp. MBC pulled the documentary, prepared for a weekly TV show called *PD Notebook*, in response to public outcry over allegations ▶

CREDIT: AHN YOUNG-JOON/AP PHOTO

that the investigative team had coerced its sources; MBC later apologized for the investigative team's transgressions. Pressian claimed that in an interview for the unaired segment, a member of Hwang's team alleged that Hwang had directed him to manipulate photographs of stem cells. The lab member had previously said that the interview was coerced. On 11 December, Hwang's team issued a statement dismissing the allegations.

In this charged atmosphere, SNU held a press conference on 12 December to announce its investigation. Jung Hye Roe, SNU's dean of research affairs, said SNU would form an investigative committee of experts from within and outside the university. They will not be publicly identified and will not respond to press inquiries. Roe said SNU may cooperate with the University of Pittsburgh, which started its own investigation at Schatten's request. One of the two SNU professors contacted by *Science* says the announcement of the investigation is welcome. But this professor added that because the details have not yet been set, "we need to keep an eye on how the investigation goes." On 9 December, *Science* Editor-in-Chief Donald Kennedy wrote to Hwang encouraging him to cooperate with efforts to verify his findings.

Colman thinks the only way to prove whether and how many of the ES cell lines match the donors is a new genetic analysis. "There is an absolute necessity now to have an independent investigator redo the fingerprinting," he says. But this could be problematic. Fresh samples might have to be taken from the donors, and that would entail again gaining informed consent. The university has not yet set any timeline for its investigation.

–DENNIS NORMILE AND GRETCHEN VOGEL

With reporting by Sei Chong, Ji-soo Kim, and Richard Stone. Chong and Kim are freelance writers in Seoul.

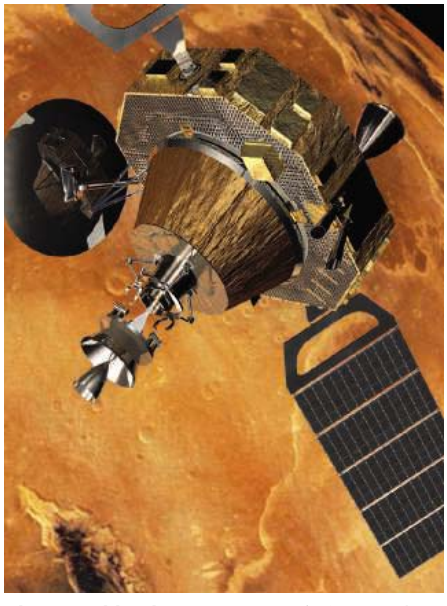
SPACE SCIENCE

ESA Hits the Right Note, and Funding Flows

To stay afloat, the European Space Agency (ESA) is forced to go through an often painful routine: It has to convene ministers from its 15 member states every few years and ask them to hammer out a long-term budget, generally requiring some hard sacrifices. But ESA got a pleasant surprise last week. Following the latest such meeting in Berlin, it came away with almost everything it asked for. ESA said it needed a total of $10.04 billion for current programs and new initiatives covering everything from launcher development to exploration of Mars; it was granted $9.87 billion, 98% of its request. There was one casualty: Ministers dropped a proposed collaboration with Russia to develop a crewed shuttle called Clipper.

The pain factor at ESA ministerial meetings usually involves haggling over how much member states are willing to pledge to mandatory programs—to which all must contribute in line with their gross domestic products—and how much each will splurge on optional programs. The largest chunk of mandatory funding goes to ESA's highly regarded science program. It has been suffering a decade-long erosion of resources as funding increases were pegged below inflation at earlier meetings. This time, science won $2.5 billion for 2006–10, which includes annual increases of 2.5%, slightly above inflation. "Psychologically, this is a very positive step," says David Southwood, the program's director.

Cost overruns in several missions over the past few years have put the science program under severe pressure. It forced the cancellation of the Eddington planet-hunting mission in 2003 (*Science*, 14 November 2003, p. 1130) and put the BepiColombo mission to Mercury under threat. The program "was facing a major crisis," says space scientist Mark Sims of the University of Leicester, U.K. Last week's reversal "makes many difficulties go away but not all of them," he adds. Southwood says the program will host a meeting of researchers in January to plan future priorities; in February, ESA's Science Program Committee will meet to decide which of four missions on the program's roster—Solar Orbiter, BepiColombo, the Gaia star-mapper, and LISA, a gravitational-wave interferometer—will get the go-ahead.



CREDIT: ESA

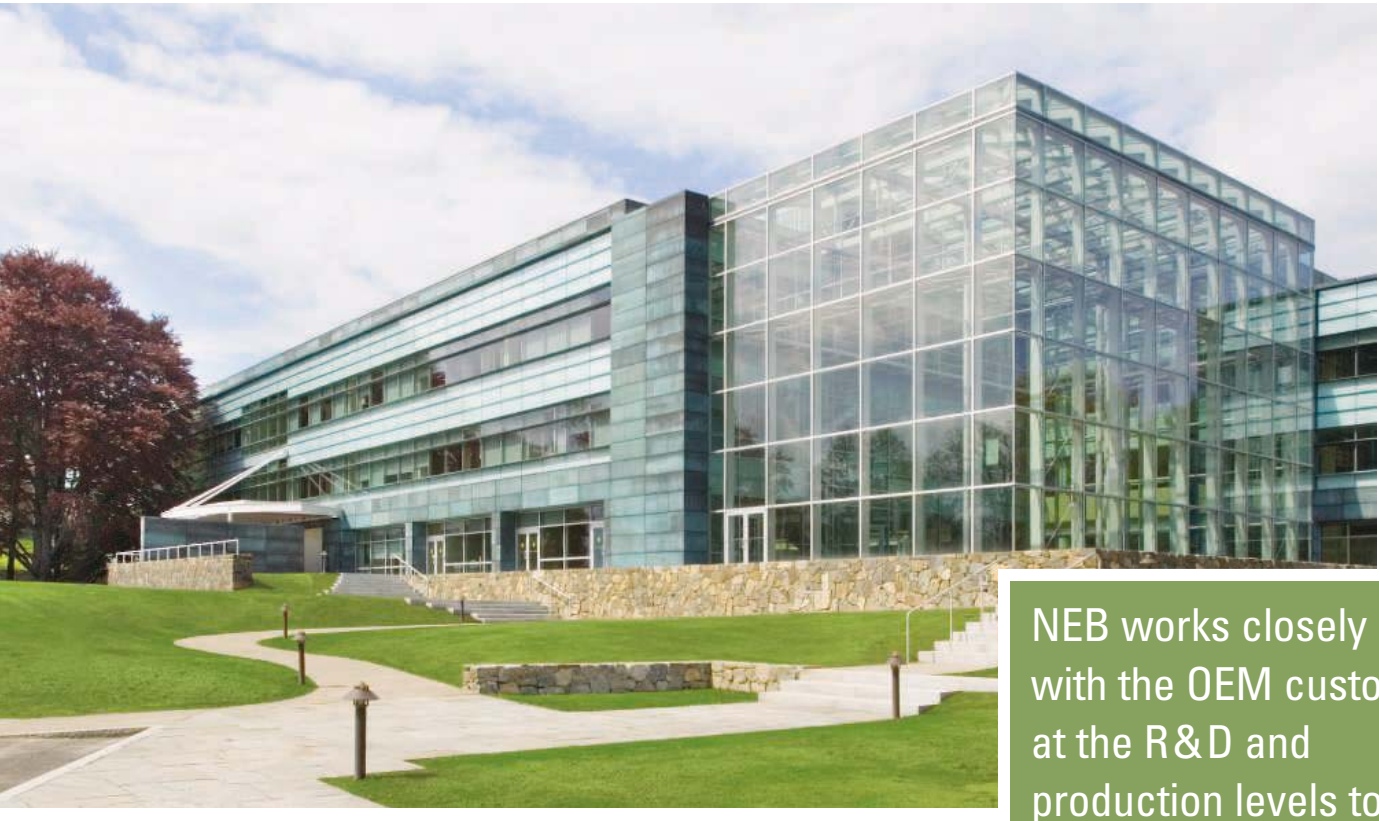
There and back again. Aurora's future plans include the Mars Sample Return mission.

The agency also won $4.3 billion to continue its programs in the earth sciences, telecommunications research, participation in the international space station, development of the new, small Vega rocket, and further refinements to the giant Ariane 5. A new program, dubbed Global Monitoring for Environment and Security (GMES), won $300 million, 26% more than ESA had asked for. GMES is a collaboration between ESA and the European Union to provide decisionmakers with environmental data from satellites.

Aurora, ESA's new optional program of planetary exploration (*Science*, 25 November, p. 1272), won enthusiastic backing. Aurora's first mission, the $700 million ExoMars, will search for signs of life on the Red Planet. It was oversubscribed by about 8% at Berlin. "This should enable the mission to be bigger" than currently planned, says Sims, who chairs the U.K.'s Aurora Advisory Committee. The extra money could pay for a small orbiter in addition to the rover and base station already planned.

The one sour note was the failure of any of Europe's large spacefaring nations—France, Germany, Italy, and the U.K.—to support Clipper. ESA asked for $60 million for 2 years of joint studies with Russian researchers to see if the minishuttle could give European astronauts independent access to space. Manuel Valls of ESA's exploration program says officials will spend the next 6 months or so refining the proposal and then present it again to member states. "It's a long-term program," Valls says. "Making it right will be worthwhile."

–DANIEL CLERY



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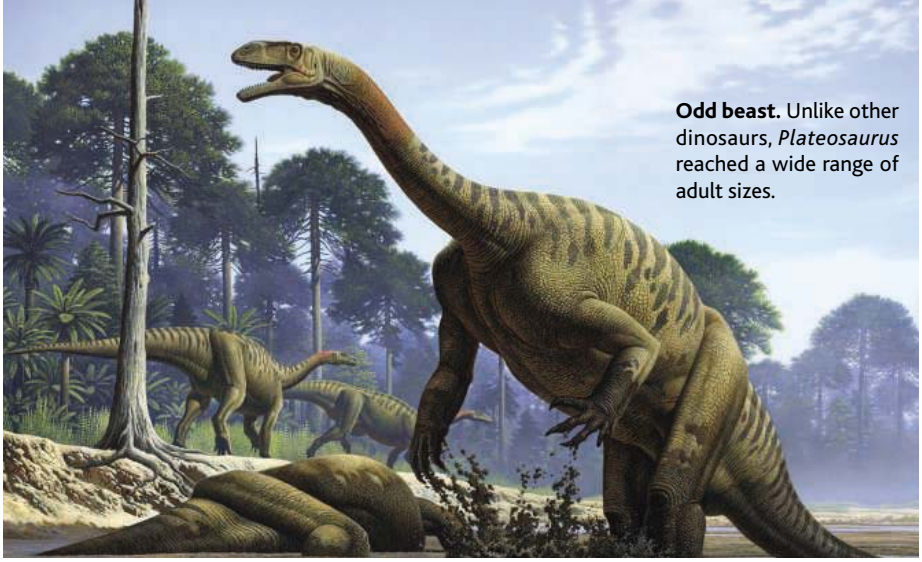


Odd beast. Unlike other dinosaurs, *Plateosaurus* reached a wide range of adult sizes.

ILLUSTRATION: RAÚL MARTÍN

PALEONTOLOGY

How Fast Does Your Dinosaur Grow?

The name may mean "thunder lizard," but dinosaurs are not actually reptiles. One key difference, paleontologists will tell you, is how fast they grew. Modern reptiles such as turtles and crocodiles grow relatively sluggishly and may reach widely different adult sizes depending on their diet and what the climate was like along the way. But studies of dinosaur bones have shown that the ancient nonlizards grew faster and attained a more or less standard adult size regardless of environmental changes—just as birds and mammals do today.

That sharp distinction has just lost its focus. New studies of bone "growth ring" patterns reveal that at least one abundant early dinosaur grew more like a reptile. The results, reported on page 1800, suggest that—in that respect, at least—the common ancestor from which all dinosaurs descended may not have been dinosaurlike at all.

"The results are very exciting," says Robert Reisz, a paleontologist at the University of Toronto's Mississauga campus in Canada. "It suggests that much of what we think of as the overall story of dinosaur evolution may have evolved independently, in different lineages."

The dino maverick is *Plateosaurus engelhardti*, a member of the prosauropods, a group of early two-legged dinosaurs that thrived from the Upper Triassic through the Lower Jurassic (about 220 million to 180 million years ago). P. Martin Sander and Nicole Klein of the Universität Bonn's Institut für Paläontologie in Germany set out to determine how it grew by scrutinizing the microscopic structure of the creature's fossilized bones—particularly a fast-growing type of bone known as fibrolamellar complex.

To distinguish faster-growing *P. engelhardti* from the slower-growing specimens, Sander and Klein counted growth rings in limb and pelvic bones from animals of similar size. Near the end of its growth phase, a slower-growing animal switches from fibrolamellar to a different kind of bone called lamellar-zonal. Full-grown specimens can be distinguished by a lack of blood vessel spaces in the bone's outer rings.

In *Plateosaurus*, that full size turned out to be highly variable, Sander says. Some animals were full-grown at less than 5 meters in length, while others grew to twice that size.

That plasticity could have evolved in either of two different ways, Sander says. In one scenario, the common "ancestral" dinosaur lacked plasticity, as later species did, but plateosaurs reverted back to an earlier, pre-dinosaurian growth pattern. In the other, the common ancestor had plasticity, and different dinosaur lineages independently evolved uniformly speedy growth rates—but plateosaurs missed the boat. So far paleontologists don't have enough fossils of other early dinosaurs to tell which way it happened, Sander says.

The finding also may help paleontologists understand how the prosauropods' more recent relatives—giant four-legged sauropods such as *Apatosaurus*—attained such enormous sizes, says Matthew Bonnan of Western Illinois University in Macomb. "The study shows that the development of the plastic rate of growth can affect maximum attainable size," he says. The challenge now, he adds, is to understand how prosauropods can shed light on the evolutionary changes that enabled sauropods to outgrow any other land animal.

The study highlights how little we still know of early dinosaur evolution, says Thomas Holtz of the University of Maryland, College Park. "There has been the tendency to infer that features found in all advanced dinosaurs were found in all of their ancestors," he says. "This emphasizes the importance of tree-based thinking. We have to look at as many branches of the evolutionary tree to get as big a picture as possible."

–CAROLYN GRAMLING

ScienceScope

Flu Defenses Bolstered

A single company has obtained the rights to a vaccine-producing technology that may prove crucial in a fight against pandemic influenza and insists it will make it widely available in an emergency. And U.S. officials have revised a vaccine policy to stretch supplies.

MedImmune in Gaithersburg, Maryland, announced last week that it has licensed patents for so-called reverse genetics from Mount Sinai School of Medicine in New York City. The company already had rights to other patents for the technology. Reverse genetics makes possible the production of seed vaccine faster and more safely than the traditional means of making seed vaccine in eggs. If MedImmune waives licensing fees for developing countries during a pandemic, as it has pledged, "there should be no downside," says infectious disease expert Andrew Pavia of the University of Utah, Salt Lake City.

Meanwhile, the Food and Drug Administration (FDA) said last month that the agency will not require that a pandemic flu vaccine containing an immune-response-boosting additive called an adjuvant go through a trial testing its efficacy at preventing infection. Instead, FDA will require only evidence of safety and an immune response to license such a vaccine. That could stretch scarce vaccine supplies in a pandemic. "It is very important that FDA has clarified its position," says Pavia. The United States is currently conducting clinical trials of vaccines with an adjuvant against the deadly H5N1 avian influenza strain that has killed more than 70 people.

–JOCELYN KAISER

NIH to Draw Cancer Map

An ambitious effort to systematically find the main genetic changes in all human cancers officially got under way this week. National Human Genome Research Institute (NHGRI) Director Francis Collins compared the effort to tackling "thousands of genome projects." The Cancer Genome Atlas (TCGA) will begin with a 3-year pilot project of $100 million in grants from the National Cancer Institute and NHGRI. Some have criticized the project as potentially futile, siphoning funds from investigator-initiated grants (*Science*, 9 December, p. 1615). To address those concerns, TCGA, previously known as the Human Cancer Genome Project, will start with just two or three tumor types and attempt to demonstrate reproducibility and clinically relevant results.

–JOCELYN KAISER

U.S. COMPETITIVENESS

Summit Lists Ways—but Not Means—to Strengthen Science

In an unusual show of unity, 50 business, academic, and legislative leaders came to Washington, D.C., last week to proclaim what they believe is obvious: The United States should be paying more attention to science and engineering. But although there was a rousing consensus on the need to improve teaching, graduate more science majors, and boost spending on research and translating the results to the workplace, there was mostly silence on how these changes might come about and who would pay for them.

The 1-day meeting, hosted by the Department of Commerce, was billed as the National Summit on Competitiveness. Although such business-oriented meetings are commonplace in the nation's capital, this one was distinguished by an intermingling of industry CEOs with university presidents, who have long lobbied for many of these changes. After a morning roundtable, the invitees attended closed sessions led by Cabinet secretaries and senior Bush Administration officials who, by several accounts, extolled the president's accomplishments in energy technology, trade, education, and research. In turn, participants maintained a relentlessly positive tone about how the United States should respond to heavy investments by other countries in their scientific workforces and high-tech industries.

"We're doing OK, but we need to do better," said Representative Sherwood Boehlert (R–NY), chair of the House Science Committee, one of the organizers of the congressionally mandated meeting. "I don't think we should be intimidated by the scope of the problem," remarked Dana Mead, chair of the MIT Corp. and former CEO of Tenneco, after moderating the morning roundtable. "Remember, the way to eat an elephant is one bite at a time."

The group's series of recommendations, announced before the meeting began, include more federal spending on basic research and set-asides for high-risk research, a doubling over the next 10 years of the number of undergraduates earning science and engineering degrees, changes in immigration laws to make it easier for foreign-born graduates to remain in the United States, and greater support for advanced manufacturing technologies. Drawn from a series of recent reports by blue-ribbon panels assembled by the likes of the National Academies, the Council on Competitiveness, and the Business Roundtable, the recommendations offer a surfeit of solutions and a dearth of details (www.usinnovation.org).

Participants made no attempt to rank the importance of those recommendations, for instance. "There are no priorities for essentials, and these are all essential," said Mead. Asked by reporters whether the Administration's signature No Child Left Behind program was likely to raise the performance of U.S. students on international science and math tests, Richard Templeton, CEO of Texas Instruments, grew testy. "The point is that we need to improve science and math education," he said. "The details are less important."

Nowhere was that hands-off approach more visible than in the summit's key recommendation to double the number of science-related bachelor's degrees awarded annually to U.S. students. The recommendation draws on testimonials from industrialists about their inability to find qualified domestic engineers for vacant positions. "My company has 180 employees, and we have 10 unfilled engineering positions," says Kellie Johnson, president of ACE Clearwater Enterprises, an aerospace and power-generation manufacturing company in Torrance, California. "Our customers are asking us to design products for them, and we can't find the right people." The recommendation also asserts that the federal government can influence the number of students pursuing such degrees by offering financial incentives such as scholarships and forgivable student loans.

But although the suggestion may seem like a no-brainer to CEOs, many educators say the situation is more complicated and that their institutions must shoulder part of the blame. The production of science, technology, engineering, and math (STEM) majors is determined by many factors, some impossible to predict, they note, and the impact of financial incentives is not clear. An annual survey of incoming freshmen, for example, shows that nearly one in three declare an interest in STEM fields, a fraction that has remained constant over the past 40 years. But only about 5% of students actually graduate with a STEM degree.

"A lot of students come to top research universities with good science backgrounds, and it takes us only 1 year to drive this interest out of them," says Thomas Cech, president of the Howard Hughes Medical Institute in Chevy Chase, Maryland. "Incentives for teachers may be a better way to go than incentives for students." In particular, poor introductory courses can discourage the most promising scientists by emphasizing rote learning over conceptual knowledge, says Alan Merten, president of George Mason University in Fairfax, Virginia.

Mark Wrighton, chancellor of Washington University in St. Louis, Missouri, and member of the National Science Board, which oversees the National Science Foundation, agrees that universities should focus on nurturing budding scientists. The former Massachusetts Institute of Technology chemistry professor says his university has decided to make research opportunities for undergraduates a priority for one simple reason: "It's so much more fun to actually do science."

Regardless of how it happens, getting more people to do science is a worthy goal, say participants. Paying for it, however, is another story. In a meeting with White House budget director Josh Bolten on the morning of the summit, Boehlert says he and two House colleagues, Representatives Vern Ehlers (R–MI) and Frank Wolf (R–VA), learned that the Administration's concern about U.S. competitiveness has its limits. "He gets it," Boehlert said about Bolten's response to the summit's recommendations. "Then he challenged us to find sources of revenue to finance these programs."

—JEFFREY MERVIS

All ears. Dana Mead moderates a roundtable discussion at last week's summit.

CREDIT: KURSTIN ROE/REFLECTIONS PHOTOGRAPHY

AFTER KATRINA

Struggling New Orleans Universities Cut Hundreds of Faculty

Faced with financial crisis, the two largest research institutions in hurricane-ravaged New Orleans are making painful cuts. Last week, Tulane University announced it will eliminate 230 faculty positions and phase out many degree programs in one of the largest-ever restructurings of a U.S. university. Louisiana State University's (LSU's) Health Sciences Center, meanwhile, has furloughed indefinitely more than 100 faculty members, some of them young researchers.

The flooding of New Orleans after Hurricane Katrina on 29 August shuttered universities and sent researchers and students to host institutions across the country (*Science*, 25 November, p. 1267). Even though 86% of its students are expected to return when the main campus opens in January, Tulane faces a budget shortfall and needs $200 million to pay for hurricane recovery. On 8 December, university president Scott Cowan announced a "renewal plan" that involves trimming weaker programs to save $55 million a year. Academic departments must lose about 50 of 500 faculty positions by May 2007, and 14 doctoral programs including sociology, economics, and several in engineering and computer science will close down.

The heaviest blow will fall on Tulane's medical school, which doesn't plan to reopen in New Orleans until next fall. It has lost income from clinical care due to the city's drastically reduced population and the closure of nine of the city's 11 hospitals. The school had hoped to receive "bridge money" from the federal government, but it didn't come through, says Paul Whelton, senior vice president for health sciences of the Tulane University Health Sciences Center. So the Tulane renewal plan calls for trimming 180 faculty positions at the center—about one-third of the total—by 31 January 2006 and focusing on the school's strengths, in infectious disease, cancer, gene therapy, organ transplantation, and heart disease. "It's a necessary action, and it's a sad one," says Whelton.

The contraction "is probably unprecedented for a research university," says William Brody, president of Johns Hopkins University in Baltimore, Maryland, who served on a panel that helped Tulane develop the plan. "It's a Hobbesian choice between two difficult decisions: Close or lose good people."

The cuts were made after department chairs compiled a list of faculty members most essential to teaching, patient care, and research, Whelton says. He adds, however, that Tulane is easing the transition by giving "very generous" separation packages with up to 1 year of paid salary for some tenured faculty. James Karam, chair of the biochemistry department, which is losing two junior professors, says he and others are hopeful that Tulane is now stable financially—the university has committed to paying salaries of remaining faculty members through spring semester 2007.

Heavy toll. Costs from Hurricane Katrina's flooding and a shrunken New Orleans population are forcing Tulane University to downsize.

A sharp drop in revenues from patient care has also devastated LSU's Health Sciences Center. On 1 December, the center placed more than 300 staff and 150 faculty members, or about 20% of the total faculty, on indefinite leave without pay. Acting chancellor Lawrence Hollier explains that the school is losing $10 million a month and could close down after February if it can't find bridge funding. Decisions about layoffs were based partly on how much independent research funding a professor had, he says.

Cell biology and anatomy assistant professor Roderick Corriveau, who says his department chair called on 21 November to tell him his last paycheck would be 9 days later, calls the furloughs "brutal." The 41-year-old developmental neurobiologist is now contacting colleagues at other institutions, trying to find spots for himself and his three graduate students. "It is like starting over," Corriveau says. "Hopefully, new doors will open."

–JOCELYN KAISER

CREDIT: KEN CEDENO/CORBIS

ScienceScope

Break the Ice, Coast Guard

A White House decision earlier this year to transfer responsibility for the U.S. icebreaking fleet from the Coast Guard to the National Science Foundation (NSF) is a bad idea. So says a National Academies' panel in an interim report released this week.

The report takes issue with the Administration's assertion that icebreaking no longer fits into the Coast Guard's mission, noting that climate change in the Arctic, for example, could bring more people to the region, adding to the Coast Guard's duties. The report also argues that NSF, as a research agency, is not equipped to manage the three icebreakers in the U.S. fleet despite its primary use in supporting scientific activity at both poles. The recommendations cover the next 4 to 8 years; a final report next summer will explore long-term options for the fleet.

–JEFFREY MERVIS

GM Protest Upheld

In a verdict last week that could undermine French agricultural biotechnology, a court in Orleans, France, acquitted 49 activists who had destroyed experimental plots planted with genetically modified (GM) maize developed by Monsanto.

The defendants had been charged with organized vandalism after ravaging two test sites near Orleans in August 2004 and July 2005. But the court agreed with their argument that the "imminent danger" of contamination of nearby crops justified the offense. In a related civil complaint, the court ordered the defendants to collectively pay $7000 in damages to Monsanto, instead of $470,000 as demanded by the company.

Environmentalists hailed the decision, but the prosecutor and Monsanto intend to appeal. "We are outraged that the court does not enforce the law," says Philippe Pouletty, chair of trade lobby France Biotech.

–MARTIN ENSERINK

Bidders Vie for Superarray

Competition is heating up for the $1 billion Square Kilometer Array (SKA) radio telescope project. Australia sent its bid this week; South Africa, China, and Argentina are due to submit before the 31 December deadline.

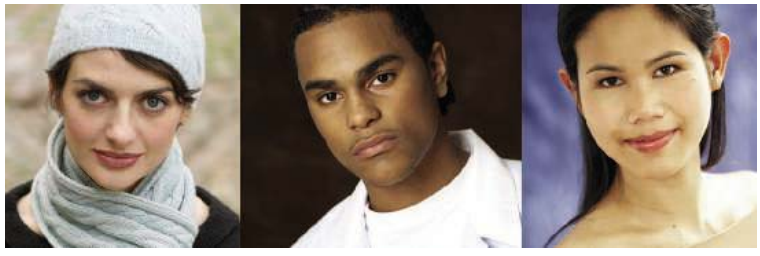
With possibly hundreds of dishes spread over a vast region, SKA will provide an unprecedented look at early galaxy formation and the nature of dark matter and dark energy. A preliminary ranking of the competing bids is expected next year, followed by a hunt for funding.

–ROBERT KOENIG

GENETICS

Zebrafish Researchers Hook Gene for Human Skin Color

People come in many different hues, from black to brown to white and shades in between. The chief determinant of skin color is the pigment melanin, which protects against ultraviolet rays and is found in cellular organelles called melanosomes. But the genetics behind this spectrum of skin colors have remained enigmatic. Now, on page 1782 of this week's issue of *Science*, an international team reports the identification of a zebrafish pigmentation gene and its human counterpart, which apparently accounts for a significant part of the difference between African and European skin tones. One variant of the gene seems to have undergone strong natural selection for lighter skin in Europeans.



Human rainbow. A newly discovered gene partly explains the light skin of Europeans, but not East Asians, as compared to Africans.

The new work is raising goose bumps among skin-color researchers. "Entirely original and groundbreaking," says molecular biologist Richard Sturm of the University of Queensland in Brisbane, Australia. Anthropologist Nina Jablonski of the California Academy of Sciences in San Francisco, California, notes that the paper "provides very strong support for positive selection" of light skin in Europeans. Researchers have not been sure whether European pale skin is the result of some selective advantage or due to a relaxation of selection for dark skin, after the ancestors of modern Europeans migrated out of Africa into less sunny climes.

Yet the authors agree that the new gene, *SLC24A5*, is far from the whole story: Although at least 93% of Africans and East Asians share the same allele, East Asians are usually light skinned too. This means that variation in other genes, a handful of which have been previously identified, also affects skin color.

The *Science* paper is the culmination of a decade of work, says team leader Keith Cheng, a geneticist at Pennsylvania State University College of Medicine in Hershey. He and his colleagues were using the zebrafish as a model organism to search for cancer genes and became curious about a zebrafish mutation called *golden*, which lightens the fish's normally dark, melanin-rich stripes. Cheng's team identified the mutated gene and found that the zebrafish version shared about 69% of its sequence with the human gene *SLC24A5*, ▶

INDIAN SCIENCE

Booming Computer Sector Seen as a Mixed Blessing

NEW DELHI—India cemented its claim to leadership in information technology (IT) last week when three U.S. companies—Microsoft, Intel, and Advanced Micro Devices (AMD)—announced plans to spend nearly $6 billion on research and manufacturing here over the next few years. The economy will benefit, but some scientists are concerned that the IT bonanza could drain talent away from basic research.

Microsoft chief Bill Gates announced on 7 December that his company will double its workforce in India to 7000 and increase its R&D investment by $1.7 billion over the next 4 years. "We depend on India for manpower, and that is why we are scaling up operations," said Gates, who unveiled plans to add a second R&D center in Bangalore to an existing one in Hyderabad.

Earlier in the week, Intel's chief executive Craig R. Barrett announced that his company will invest $1 billion over the next 5 years, including $200 million for development of a microprocessor being researched at its center in Bangalore. AMD is investing $3 billion in a chip-manufacturing plant at an undisclosed location.

Great expectations. Microsoft Chair Bill Gates meets with India's Minister of Information Technology, Dayanidhi Maran.

According to the National Association of Software and Service Companies (NASSCOM) in New Delhi, Indian software and services exports grew more than 34% from 2004 to 2005, earning revenues of $17.2 billion over a 12-month period. India attracts IT companies, NASSCOM argues, because it has a well-educated English-speaking workforce, low labor costs, and a time zone that allows Western companies to run operations around the clock.

Although the IT sector is booming, some leaders fear that its rapid growth could hurt other areas of research. Astrophysicist Rajesh Kochhar, former director of the National Institute of Science, Technology, and Development Studies in New Delhi, says: "There can be no doubt that information technology is acting as a brain sink." New entrants in the Indian IT sector are paid roughly three times as much as entry-level scientists, he says. The result, he argues, is that "highly qualified engineers are doing stupid, repetitive work." Echoing this view, aeronautics engineer Gangan Prathap, chief of the Centre for Mathematical Modelling and Computer Simulation in Bangalore, says foreign investments like those announced this week could "seduce" Indians into becoming "a nation of techno-coolies." He claims that academic centers already must "scrounge at the bottom of the barrel" for talent.

Other science community leaders take a more optimistic view. M. Vidyasagar, executive vice president of software company Tata Consultancy Services in Hyderabad, dismisses internal brain-drain concerns as nothing more than "disguised envy." And Raghunath Anant Mashelkar, a polymer engineer and president of the Indian National Science Academy in New Delhi, says there is undoubtedly "a war for talent at the top of the ladder." But if it leads to a stronger economy, he thinks that both commercial R&D and basic science will benefit. —**PALLAVA BAGLA**

CREDITS (TOP TO BOTTOM): PHOTOS.COM; P. BAGLA

which is thought to be involved in ion exchange across cellular membranes—an important process in melanosome formation. And when Cheng and his co-workers injected human *SLC24A5* messenger RNA (an intermediary molecule in protein synthesis) into *golden* zebrafish embryos, wild-type pigmentation pattern was restored.

Researchers say the ability of human *SLC24A5* to "rescue" the mutant zebrafish is strong evidence that the gene has a similar function in fish and humans. "The zebrafish data are extremely compelling," says human geneticist Neil Risch of the University of California, San Francisco.

The team then searched for genetic variants among humans. Data from the HapMap database of human genetic diversity (*Science*, 28 October, p. 601) showed that *SLC24A5* has two primary alleles, which vary by one amino acid. Nearly all Africans and East Asians have an allele with alanine in a key locus, whereas 98% of Europeans have threonine at that locus. These marked frequency differences combined with the pattern of variation in nearby genes suggest that the threonine variant has been the target of a recent selective sweep among the ancestors of modern Europeans, Cheng's team concluded.

Finally, the team measured the pigmentation levels of 203 African Americans and 105 African Caribbeans—groups that represent an admixture of African and European ancestry—and compared their *SLC24A5* genotypes. Subjects homozygous for the threonine allele tended to be lightest skinned, those homozygous for the alanine allele were darkest, and heterozygotes were in between, as shown by the degree of reflectance of their skin. The team concludes that between 25% and 38% of the skin-color difference between Europeans and Africans can be attributed to *SLC24A5* variants. The experiments provide "a beautiful example of the critical role that model organism genetics continues to play for understanding human gene function," says geneticist Gregory Barsh of Stanford University in California.

The new work doesn't solve the question of why fair skin might have been favored among Europeans. However, it is consistent with a long-standing but unproven hypothesis that light skin allows more absorption of sunshine and so produces more vitamin D, a trait that would be favored at less sunny European latitudes.

Barsh adds that the paper "indicates how the genetics of skin-color variation is quite different from, and should not be confused with, the concept of race." Rather, he says, "one of the most obvious characteristics that distinguishes among different humans is nothing more than a simple change in activity of a protein expressed in pigment cells." Jablonski agrees: "Skin color does not equal race, period." —MICHAEL BALTER

SCIENTIFIC PUBLISHING

Echoing Other Cases, *NEJM* Says Vioxx Safety Data Withheld

When the *New England Journal of Medicine* (*NEJM*) last week released a scathing editorial asserting that a study on Vioxx had omitted safety data, the episode became the latest chapter in the efforts of medical journal editors to keep what they consider misleading drug studies from their pages. The editorial contended that the authors of the influential 2000 study in *NEJM* failed to report three out of 20 heart attacks among patients treated with Vioxx and data on cardiovascular ailments such as angina.

A string of similar cases have prompted journals to tighten requirements of authors, ask increasingly pointed questions before publishing, and require that clinical trials be publicly registered before papers are reviewed. Yet those measures may not be enough, say editors. "We now hold [a paper] up to the light and say, 'This seems like a very well done study; can we believe it?' " says Drummond Rennie, a deputy editor at the *Journal of the American Medical Association* (*JAMA*). "What can we do? … We can't go wired into their lab."

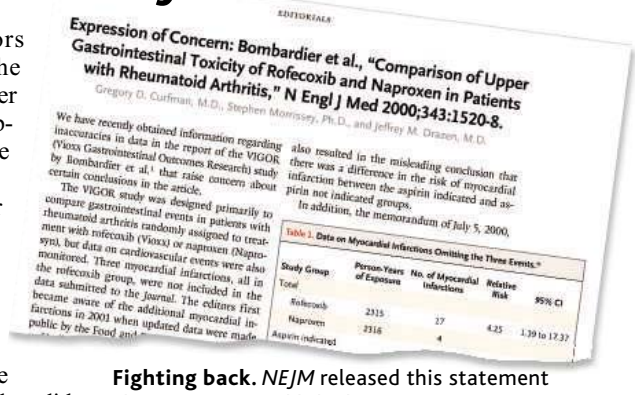
The latest case came to light when Gregory Curfman, an *NEJM* editor, was deposed on 21 November in the third Vioxx lawsuit. (The jury deadlocked, producing a mistrial this week.) Curfman learned from a Merck memo of three unreported heart attacks, which he realized had been deleted from a paper comparing the gastrointestinal effects of Vioxx with those of the anti-inflammatory naproxen, says Karen Pedersen, an *NEJM* spokesperson. (Curfman was not available for comment.) Data showing other cardiovascular problems were removed just 2 days before the manuscript was submitted, according to *NEJM*. Pedersen says the journal's editors crafted their editorial, sent it to the paper's lead author Claire Bombardier of the University of Toronto, and published it online. They also invited the authors to submit a correction.

In an e-mail to *Science*, Bombardier said that she and the other authors are preparing a reply to *NEJM* and declined to comment until that's complete. In a statement, Merck denied any wrongdoing, asserting that the three heart attacks occurred after the study's prespecified completion and thus did not warrant inclusion. The company also noted that the heart attacks were disclosed to the Food and Drug Administration.

This new Vioxx flap produced "flashbacks," says Christine Laine, senior deputy editor of the *Annals of Internal Medicine*. Last spring, her journal learned from a reporter that a 2003 Vioxx paper reporting several heart attacks excluded a sudden cardiac death. Because the paper was not technically in error—the cardiac death was not necessarily due to a heart attack—the journal published only a letter from the Merck co-authors. As part of its detailed author questionnaire, the *Annals* now asks whether a professional or industry writer was involved in the paper. And rather than simply asking authors what contributions they made to the research, the journal inquires at which stage they became involved.

JAMA, which was also singed by a COX-2 inhibitor paper it published in 2000, now insists on an independent statistical analysis of raw data from clinical trials and uses a questionnaire that's increasingly specific, querying the authors about their separate contributions. The International Committee of Medical Journal Editors, a consortium of 12 medical journals and the U.S. National Library of Medicine, has also tried to tighten guidelines around conflict-of-interest disclosure and press its members to publish more negative trials.

In September, the consortium, which includes *JAMA*, *NEJM*, and *Annals*, began requiring registration of clinical trials before it would consider publishing them. The goal is to ensure that reported results conform to the trial's design, and that there is a public record of trials whose results go unreported—often because the findings are negative. At the National Institutes of Health's ClinicalTrials.gov, the number of trials registered shot from 12,000 in the spring to more than 30,000 today. "It really looks like the policy … had a big impact," says Deborah Zarin, director of the database. —JENNIFER COUZIN



EDITORIALS

Expression of Concern: Bombardier et al., "Comparison of Upper Gastrointestinal Toxicity of Rofecoxib and Naproxen in Patients with Rheumatoid Arthritis," N Engl J Med 2000;343:1520-8.

Gregory D. Curfman, M.D., Stephen Morrissey, Ph.D., and Jeffrey M. Drazen, M.D.

We have recently obtained information regarding inaccuracies in data in the report of the VIGOR (Vioxx Gastrointestinal Outcomes Research) study by Bombardier et al.,[1] that raise concern about certain conclusions in the article.

The VIGOR study was designed primarily to compare gastrointestinal events in patients with rheumatoid arthritis randomly assigned to treatment with rofecoxib (Vioxx) or naproxen (Naprosyn), but data on cardiovascular events were also monitored. Three myocardial infarctions, all in the rofecoxib group, were not included in the data submitted to the journal. The editors first became aware of the additional myocardial infarctions in 2001 when updated data were made public by the Food and

also resulted in the misleading conclusion that there was a difference in the risk of myocardial infarction between the aspirin indicated and aspirin not indicated groups.

In addition, the memorandum of July 5, 2000,

Table 3. Data on Myocardial Infarctions Omitting the Three Events.*

Study Group	Person-Years of Exposure	No. of Myocardial Infarctions	Relative Risk	95% CI
Total				
Rofecoxib	2315	17	4.25	1.39 to 12.37
Naproxen	2316	4		
Aspirin indicated				

Fighting back. *NEJM* released this statement about a paper it published.

With "mad cow disease" declining sharply, public anxiety about prion diseases has diminished. But cutting funds would be a big mistake, prion researchers say

After the Crisis: More Questions About Prions

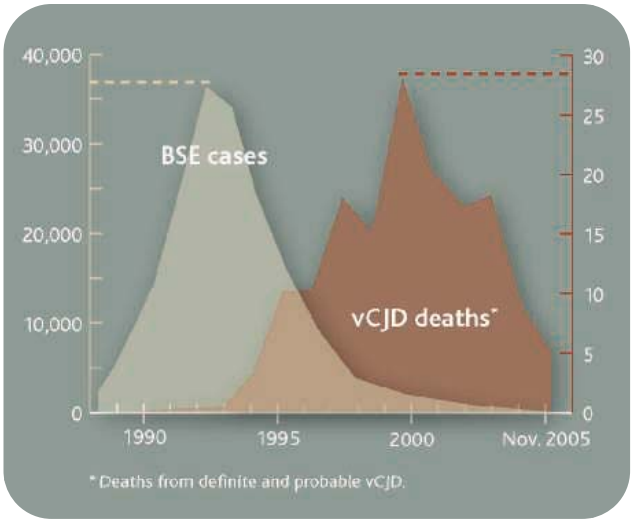
DÜSSELDORF, GERMANY—Peering over an audience of more than 700 researchers on 19 October, Nobel laureate Stanley Prusiner seemed pleased. "This is probably the largest gathering of prion scientists ever," boasted the field's controversial godfather, who gave the keynote speech at a recent meeting.* As the crowd attested, prion science had come a long way since Prusiner proposed a heretical idea 23 years ago that it is not viruses or bacteria, but weird proteins, that cause a family of lethal brain diseases.

But now, leaner times may be ahead. Public health efforts to combat prion infections in cattle have worked so well that reports about "mad cow disease" have all but vanished from the newspapers; the clamor for action is fading, and governments are looking for ways to scale back costly safety measures. And many worry that research may suffer; trimming has begun in Germany and France. Prusiner captured the atmosphere best in a private quip after his keynote speech, according to conference organizer Detlev Riesner of Heinrich Heine University, when he said the largest prion meeting to date could end up being the largest in history.

Prion researchers admit there's reason to breathe a little easier. Outbreaks of mad cow disease, or bovine spongiform encephalopathy (BSE), have declined ever since reaching a peak in the United Kingdom, by far the hardest-hit nation, in 1992. Fears of a massive wave of an associated human brain disease called variant Creutzfeldt-Jakob disease (vCJD) have not materialized.

But a slowdown in research would be the wrong response, prion scientists say. The British vCJD outbreak could still be in its infancy, and medical procedures could trigger a second wave. (Tests to screen blood, organs, and tissue are still some time away.) There are other reasons to stay alert as well. Europeans have reported the appearance of a new form of scrapie, an age-old prion disease in sheep. And a prion disease in North American deer and elk is spreading rapidly. "The fire is out, but there are still glowing red spots everywhere," says Jean-Philippe Deslys, head of the prion research group at the French Atomic Energy Commission.

And leaving aside public and animal health, researchers say their field has barely begun to crack its mysteries.



Twin peaks. Both mad cow disease (BSE) and human variant CJD cases have declined sharply in Britain. But some experts warn that vCJD could bounce back.

Debatable

Even after decades of research, the most fundamental question about the prion family of diseases remains open: What is the infectious agent? Many researchers today say recent experiments have convinced them that Prusiner's dogma-defying theory is correct: A rogue protein imposes its own misfolded shape on other, healthy proteins—but some still have doubts (see sidebar on p.1758).

And other riddles remain. For example: After oral infection, how do prions travel from the gut to the brain? They are known to pass through lymphoid tissue and peripheral nerves, but do individual misfolded proteins make that journey, or do they infect their neighbors, causing them to fall like dominoes? Once present in the brain, misfolded proteins form aggregates that appear to be involved in killing neurons. But exactly how is unclear.

Fortunately, answers to these questions weren't needed to start bringing the BSE and vCJD epidemics under control. Primarily as a result of a 1988 ban on feeding so-called rendered protein, including brain tissue, from ruminants to ruminants, the number of BSE cases in the United Kingdom began to fall in 1993; there were only 343 last year and just 151 so far in 2005 (see graphic). Other countries in Europe, after discovering about the year 2000 that they had their own BSE problems, now report rapid declines, too.

In reaction, the European Union (E.U.) is beginning to loosen measures to stop BSE and limit human exposure. A "road map" for prion diseases, published by the European Commission in July, listed restrictions that might eventually be lifted, arguing that

* Prion 2005. Between Fundamentals and Society's Needs. Düsseldorf, 19–21 October.

CREDITS (TOP TO BOTTOM): AFP/GETTY; SOURCES: OIE (BSE); NATIONAL CJD SURVEILLANCE UNIT (VCJD)

resources should be concentrated on new health threats such as avian influenza. (Testing of apparently healthy animals at the slaughterhouse cost about €1.6 billion between 2001 and 2004—€1.6 million per BSE case detected.)

And in October, the commission delighted lovers of T-bone steak and other meat on the bone by raising the age from 12 to 24 months at which the vertebral column—one place where prions concentrate—is removed. (Generally produced from cattle aged 22 to 30 months, such cuts had virtually disappeared.) That decision was premature, says Martin Groschup of the Friedrich Löeffler Institute, Germany's federal animal health center. His lab is still carrying out a long-term BSE pathology study to discover at what age and where in the cow's body infectious particles collect; the decision should have been stayed pending the outcome, he says.

Thanks in part to the decline of BSE, more scientists are now turning their attention to sheep. Scrapie has been known to infect flocks for at least 250 years and is harmless to humans. But in the lab, sheep can also be infected with BSE. Researchers have long worried that the resulting disease—simply called "BSE in sheep"—could get into Europe's flocks, for instance, through feed. If it were transmissible among sheep, like scrapie, it would pose a special problem because a feed ban would not get rid of it, says Lucien van Keulen of the Central Institute for Animal Disease Control in Lelystad, the Netherlands. But so far, there's no evidence of this.

The increased surveillance has turned up a new problem, however. In the last 3 years, researchers in Germany, Portugal, and France have discovered a new variety of scrapie whose prion proteins accumulate in different parts of the brain, have different biochemical properties, and produce a slightly different set of symptoms. Most likely, says Groschup, it's a variant of scrapie that flew under the radar until now. What's disconcerting is that it also appears to affect sheep with a genotype called ARR/ARR, thought to confer resistance to scrapie. Now, some worry that an ambitious E.U. breeding program aimed at spreading that genotype could just replace classical scrapie with a new form. "It's another thing we need to get to the bottom of," says Neil Cashman of the University of British Columbia in Vancouver, Canada.

Meanwhile, in the United States and Canada, chronic wasting disease (CWD), first discovered in deer and elk in Colorado and Wyoming in the 1980s, keeps turning up in new places. In 2005, New York became the 13th state affected, and moose the fourth species. So far, there is no evidence that CWD can cross the species barrier to humans—nor, for that matter, nonmembers of the deer family. CWD hasn't appeared in Europe, but the E.U. is planning a survey in 2006 to make sure.

Deceptive calm?

In BSE's wake, vCJD is declining too; there were just nine deaths last year in the United Kingdom, down from 28 in 2000 (see graph), and the total death toll stands at 153 (plus fewer than 20 in other countries), far below worst-case predictions in the late 1990s.

Old news. Concern about vCJD cases made headlines in the 1990s. Now that the crisis seems to be over, some public health and research measures are being scaled back.

But some believe the curve may be deceptive. John Collinge of the National Hospital for Neurology and Neurosurgery in London notes that vCJD's peak came barely 10 years after the highest BSE exposure in Britain. The delay is just too short, he says. Kuru, a disease among the Fore people in the highlands of New Guinea that resulted from cannibalistic rituals in the 1950s, has a mean incubation period of about 12 years. BSE ought to take longer, Collinge says, because in all known instances, crossing a species barrier lengthens a prion disease's incubation period.

Collinge suggests another possibility: Only the most genetically susceptible people have developed symptoms so far. Researchers know that having the "wrong" amino acid at codon 129 of both copies of the prion gene makes a person more susceptible to vCJD. All patients so far except one, who likely contracted vCJD through a blood transfusion, had this genotype, called MM. But other genes may be involved as well, says Collinge; the victims so far may just be an especially susceptible vanguard of the MM population at large, which comprises 40% of U.K. residents.

The possibility that many more people harbor the disease without symptoms—and the fact that probable vCJD transmission through blood transfusions has now been shown twice—means that, rather than slacking off now, efforts to develop drugs and diagnostic techniques should be intensified, Riesner says. At the meeting, several groups reported encouraging data that could lead to a blood test within the next several years. Drug development has been slower, in part because the pharmaceutical industry has little interest in a disease that affects about one in a million people.

Researchers have tried at least half a dozen compounds on CJD patients, but most seem to prolong life by only a few weeks—if they do anything. An ongoing U.K. trial of a drug called quinacrine for vCJD and CJD, in which 53 patients have been enrolled, is primarily a way to discover how to run future tests, says Collinge, whose group is one of three mass-screening small compounds in vitro in a search for promising new candidates.

Because of the countless remaining questions, many scientists say they worry about the unmistakable decline in public interest. Cashman, for instance, says he was amazed a "media firestorm" didn't break out after a paper in the October issue of *Nature Medicine* showed that prions can lurk in the inflamed mammary glands of scrapie-infected sheep—and presumably their milk as well. If the same is true in cows, he said, "it would be a hugely important finding for public health."

So far, funding doesn't appear under threat in the United States or the United Kingdom, and it is even expanding in Canada. Three weeks ago, the Canadian government announced a new U.S. $30 million network of centers of excellence; sepa-

CREDIT: TOUHIG SION/CORBIS

Waiting for the Final Experiment

The Nobel Committee went out on a limb in 1997, some biologists thought, when it awarded science's highest honor to neurologist Stanley Prusiner of the University of California, San Francisco. Prusiner had championed the idea that a mysterious class of infectious particles called prions consisted of nothing but protein. Even some who thought he was on the right track wanted more evidence.

The theory has stronger support today. Some, like Detlev Riesner of Heinrich Heine University in Düsseldorf, Germany, say papers published in the past 18 months, including one by Prusiner, have nailed the case for infectious proteins. "It's beyond any doubt now," says Riesner. But not everyone agrees. A few researchers believe Prusiner is spectacularly wrong; many more say the evidence is getting stronger but isn't irrefutable yet.

The "protein-only hypothesis," as it's often called, holds that the infectious agent in prion diseases consists of an abnormally folded protein, PrP^{Sc}, with a bizarre power over its neighbors. It can impose its own three-dimensional shape on an abundant protein in mammalian cells (called PrP^{C}) that has the same amino acid sequence but a different structure. The altered proteins then help recruit more PrP^{C}, according to theory, and over the years the chain reaction causes large amounts of PrP^{Sc} to build up in the brain and cause death. No bacterium or virus is needed to accomplish this.

Yale researcher Laura Manuelidis is among the people who think this scenario is all wrong. For decades, she has advocated the notion that the true culprits in prion diseases are slow-acting, elusive viruses. That would explain far better why so-called strains of prion diseases with slightly different characteristics have been found, she says. Manuelidis published a paper in *Science* in October showing that infection with a slow-acting Creutzfeldt-Jakob strain protects mouse cells from infection with a faster one—a finding she says points to an immune defense reaction and thus a virus. But many researchers say her results can also be interpreted within the protein-only theory. Although Manuelidis's studies are good, says Riesner, "her conclusions are wrong."

The experiment that could irrefutably prove Prusiner right, meanwhile, is easy on paper but difficult to perform, says Byron Caughey of the U.S. National Institute for Allergy and Infectious Diseases lab in Hamilton, Montana: Synthesize PrP^{Sc} in vitro and show that it can, by itself, produce an infectious disease in healthy animals. Several labs have tried to do this and failed, leading to renewed speculation that something other than proteins is involved after all, Caughey says.

Prusiner and his team reported last year in *Science* (30 July 2004, p. 673) that they had created such "synthetic prions." The group engineered *Escherichia coli* bacteria to produce part of a mouse prion protein, polymerized it into misfolded fibrils akin to PrP^{Sc}, and injected these into the brains of mice, where they triggered a neurodegenerative disease that could be transmitted to other animals.

The work won over Riesner, but other researchers saw problems. Prusiner's mice were engineered to express 16 times the normal amount of prion protein, which could lead them to develop prions spontaneously, Caughey says. The "very important control" to show that they don't is missing.

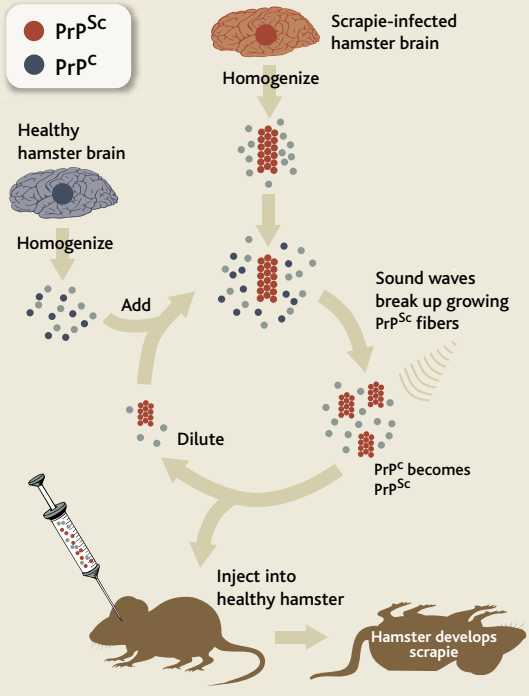
Meanwhile, a group led by Claudio Soto of the University of Texas Medical Branch in Galveston has tried a different tack. Building on earlier work by Caughey, Soto developed a technique called protein misfolding cyclic amplification (PMCA), which can multiply PrP^{Sc} in the test tube. In PMCA, the brain of a hamster infected with a prion disease called scrapie is ground up till it becomes a cell-free soup called a homogenate; when a similar brain homogenate from a healthy hamster is added, PrP^{Sc} from the sick brain will transform any PrP^{C} to PrP^{Sc}. (The test tubes are blasted periodically with a short sound wave to break up growing PrP^{Sc} fibers.) The mixture is diluted into more healthy brain homogenate, and the process is repeated.

In a *Cell* paper published in April, the group showed that even after hundreds of cycles and a 10^{20}-fold dilution—meaning not a single molecule of the original sick brain was left—the reaction produced PrP^{Sc} that sickened healthy hamsters. The study demonstrates that molecules made entirely in vitro and free of viruses—which can't live without cells—can generate infection, Soto says.

Although it's a "fantastic result," Caughey says, the study doesn't clinch the case for the protein-only theory. Because the reaction takes place in a complex, brain-derived chemical mix, one cannot rule out that, say, a small piece of nucleic acid that's essential to infectivity was replicated along with PrP^{Sc} in each cycle. Soto says that's unlikely. Nonetheless, he is now planning experiments in which the PMCA process is fed with purified PrP^{C} rather than brain homogenate. He believes that should dispel the skepticism once and for all.

–M.E.

Protein Misfolding Cyclic Amplification



Prion factory. By mixing scrapie-infected brain material with healthy brain in a process called PMCA, researchers say they made infectious proteins in a test tube.

CREDIT: K. BUCKHEIT/*SCIENCE*; SOURCE: C. SOTO

rately, the government of the province of Alberta has committed $33 million to launch a prion research institute. The reason: Canada recently learned how devastating prion diseases be. Four cases of BSE since 2003 have cost the economy an estimated $5.5 billion. (As Cashman puts it, "those cattle might as well have been space shuttles—they cost the same.")

But in Germany, prion projects worth about €10 million, funded by three federal ministries since 2001, will come to an end in 2006; they include the German Transmissible Spongiform Encephalopathy Research Platform, which coordinates studies and sample sharing through three depositories. Several German states' programs will end next year as well, says Kerstin Dressel, the platform's scientific secretary. In France, funding is set to decline as well, Deslys says.

Still, not everyone is worried. If it turns out that after BSE, prion diseases pose no major new health risks, well, "then it would only be natural that the money goes elsewhere," says Byron Caughey, a veteran prion researcher at the U.S. National Institute of Allergy and Infectious Diseases Rocky Mountain Laboratories in Hamilton, Montana. "Then we'll have to adapt, as scientists do."

–MARTIN ENSERINK

Hawaii's Coral Trees Feel the Sting of Foreign Wasps

Island researchers are desperate to find a natural enemy of the parasitic wasps that are killing a local treasure, the wiliwili

The once-beautiful coral trees on the University of Hawaii's Manoa campus where botanist David Duffy works have deformed lumps where the leaves and flowers should be. "Trees here look like they have been hit by a flamethrower," says Duffy.

The bulbous growths are infested with tiny wasps, a recently identified parasitic species that first appeared in southern Taiwan in 2003. Within a year, the wasps had spread across the island and had also reached Singapore, Reunion, and Mauritius. By 2005, they appeared in Hong Kong and China and were first seen on Hawaii's Oahu island this April. By August, the wasps had invaded every island in the state, threatening the existence of one of Hawaii's most enduring symbols, a native tree locally known as the wiliwili that provides flowers and seeds for leis and bark for canoes.

Threats to Hawaii's native species by foreign invaders are nothing new. Long cut off from the rest of the world, Hawaii's endemic species are particularly vulnerable to invasion by foreign insects, plants, and other organisms, and state officials constantly race to keep up with the latest threat (*Science*, 2 December, p. 1410). But even the state's weary conservationists have been stunned by the speedy efficiency with which this latest pest has spread from island to island. And now, researchers are struggling to identify any measure, from burning infested trees to chemical or biological defenses, that can halt the wasps' devastation of the native wiliwili and other nonnative species of coral trees. "Either all the trees are going to die, or they'll never be the same again," says botanist Art Medeiros of the U.S. Geological Survey's Haleakala Field Station on Maui.

The wasps, dubbed *Quadratichus erythrinae* in 2004, lay their eggs in green stems and leaves of the trees, creating outbreaks of tumors that stunt the trees' growth and eventually kill them. The wasps disperse easily as larvae-infested tissue falls off and is scattered with the wind, or as adult wasps emerge to lay more eggs in new growth.

Inside attack. Infestations of parasitic wasps (*right*) transform Hawaii's cherished coral trees (*top*) into deformed eyesores (*bottom*) and threaten their survival.

The Asian-Pacific path of the wasps parallels the habitat of the genus of trees called *Erythrina*, popularly known as coral trees. *Erythrina*'s 115 species are found around the world in tropical and warm temperate regions, from Southeast Asia to the southeastern United States. With their bright red flowers, they are highly prized as ornamental trees and have figured widely in local mythology. Native and nonnative *Erythrina* are both extensively cultivated in Hawaii, but the wiliwili is the only species of the tree that is found exclusively in the state. A dominant species in the large dry forests that form on the leeward slopes of many of the islands, wiliwili grow on rocky lava substrates called aa, a forbidding terrain that has helped discourage previous invaders.

"The species has been bulletproof," says Medeiros. But now, he fears, the trees are in danger of extinction.

That danger has resulted in a concerted effort by state and federal officials and university researchers to find an effective remedy. Cutting down infested trees and burning the detritus has proved ineffective, Duffy says: The wasps simply spread too quickly. Another possible solution is injecting the trees with an insecticide, says Anne Marie LaRosa of the U.S. Department of Agriculture's Forest Service in Hilo. However, such a strategy is very expensive, costing nearly $30 per tree, and is likely to prove impractical on trees in the wild.

"The only point in treating them chemically is as a stopgap method," LaRosa says. Injections could preserve some trees for a while, giving researchers more time to identify a biological control agent—now considered the only viable long-term solution.

But biological control agents are fraught with their own dangers, as Hawaii well knows. Fifty years ago, a different species of parasitic wasp was brought into Hawaii to repel sugar-cane pests; those wasps now dominate the food web of the Alakai Swamp, a wilderness preserve on Kauai island. Such cautionary tales highlight the need for stringent prerelease testing to ensure that the new agents won't run amok, researchers say (*Science*, 17 August 2001, p. 1241). "We need to be incredibly sure that whatever we try to introduce will not attack native species in Hawaii," says entomologist Daniel Rubinoff of the University of Hawaii, Manoa.

Because the coral tree wasp is brand-new, adds Rubinoff, he and other researchers seeking a biological control agent have their work cut out for them. Rubinoff, with colleagues Russ Messing and Mark Wright, is working on identifying the origin of the wasp. Africa is the likeliest source, they believe: Scientists in South Africa have seen similar gall-forming parasitoid wasps on *Erythrina* species in the region. As a result,

CREDITS (TOP TO BOTTOM): ART MEDEIROS; RON HEU/WALTER NAGAMINE; MICHELLE TREMBLAY (INSET)

Hawaiian researchers are soliciting wasp samples from colleagues in Kenya and South Africa, and they are preparing to mount expeditions to other possible hot spots on the continent, hoping to locate a natural enemy that will be specific to the wasp.

The University of Hawaii team will head to South Africa in March 2006, which should coincide with the end of the rainy season there, when the trees will be sporting new growth and infestations will be easier to find. Meanwhile, state of Hawaii entomologist Mohsen Ramadan hopes to leave by the end of 2005 for Tanzania, also to coincide with the rainy season in that country.

Back in Hawaii, scientists are working on a last-ditch solution. Called the "Noah of wiliwili," Alvin Yoshinaga, a botanist at the University of Hawaii's Center for Conservation Research and Training in Honolulu, is overseeing a collection of the trees' seeds, harvested by volunteers on all the islands and hoarded against the day that a wasp-control method is found. "We are trying to gather seeds from as many subpopulations on different islands as possible," Duffy says. Identifying an effective but safe biological control agent could take anywhere from 1 to 50 years, he adds—and the trees almost certainly wouldn't last that long.

"We have very little time," Rubinoff agrees. "All of the *Erythrina* are being hammered."

–CAROLYN GRAMLING

Meeting Environmental Epigenomics

Food, Tobacco, and Future Generations

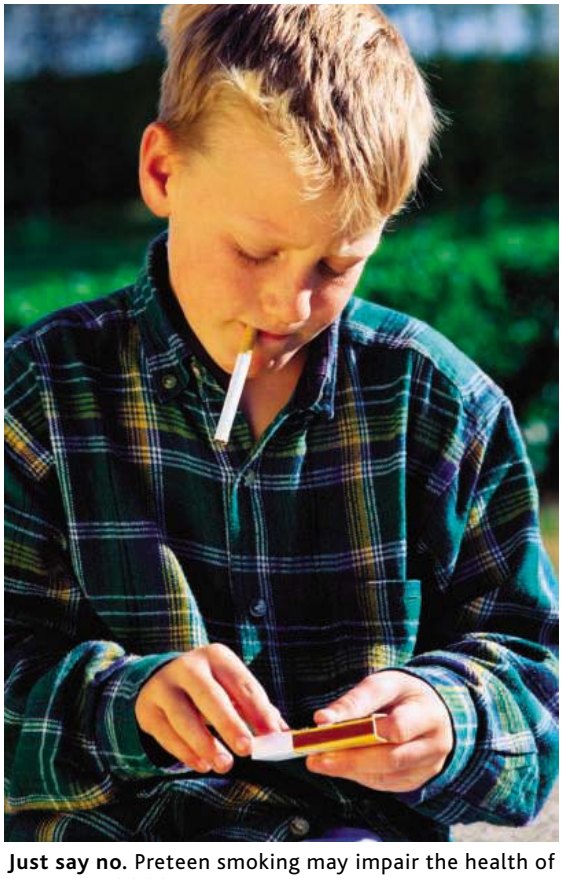
DURHAM, NORTH CAROLINA—Geneticists, molecular biologists, and epidemiologists discussed epigenetics from 2 to 4 November at the Environmental Genomics, Imprinting, and Disease Susceptibility conference.

One day, doctors taking family histories may ask not just about patients' diet and smoking habits, but also about their parents' and grandparents' food and tobacco consumption. The reason: There's increasing evidence that a person's health may be influenced by the lifestyle of past generations.

At the meeting, Marcus Pembrey, a geneticist at University College London, offered two new studies supporting this surprising link. He reported that a man's taste for tobacco as a boy appears to increase the risk that his sons will be overweight as children. In a second study, Pembrey and his Swedish colleagues found that a person's risk of early death, and in some cases, diabetes, is influenced by the eating patterns of their paternal grandparents. (The results of both studies appeared online 14 December in the *European Journal of Human Genetics*.)

Pembrey says the mechanism is unclear, but it may be that certain eating patterns or smoking at critical periods in life cause epigenetic changes—chemical modifications of a gene's DNA rather than direct mutations—that can silence genes in sperm and eggs. These changes may persist for more than one generation. These two epidemiological studies "highlight the profound impact of our behavior on the health of future generations," says Moshe Szyf, an epigeneticist at McGill University in Montreal, Canada.

Pembrey's evidence for the effects of smoking comes from the Avon Longitudinal Study of Parents and Children. This long-term U.K. study enrolled about 14,000 pregnant women almost 15 years ago and has tracked lifestyle, diet, growth, and disease in these women's families. Because the study included data on smoking, Pembrey decided to look at whether tobacco consumption influenced transgenerational health outcomes. About 5400 fathers in the database were smokers; most had taken up the habit by age 16. (There were too few women smokers to study.)



Just say no. Preteen smoking may impair the health of future grandchildren.

CREDIT: GETTY IMAGES

Pembrey and his colleagues examined whether there was any connection between when a father had begun smoking and his children's weight at age 9, a measurement included in the Avon study. There were 166 fathers who started smoking before age 11, and Pembrey found that these fathers' sons were on average heavier than sons of fathers who took up this habit later in life or who never smoked. To his surprise, daughters were unaffected. "This is the first report of an acquired parental exposure, smoking, influencing metabolic processes in sons but not daughters," says Bruce Richardson, a geneticist at the University of Michigan, Ann Arbor.

In another effort to pinpoint transgenerational risk factors, Pembrey reanalyzed data from a provocative 2002 study in which Swedish researchers had delved into more than a century of birth, death, health, and genealogical records on 300 Swedish families in an isolated village. This rich data set also included crop records and food prices. The Swedish team determined that the grandchildren of individuals who enjoyed a surplus of food during childhood had a higher risk of diabetes than those whose grandparents grew up in times of food scarcity.

When Pembrey and his Swedish colleagues looked more closely at these data, they found that these effects were sex-specific. The health of grandsons, but not granddaughters, was related to the food supply of their paternal, but not maternal, grandfathers. And the health of granddaughters was tied only to that of paternal grandmothers, Pembrey reported.

As in the case of smoking, timing seems to be critical. Food surpluses during a paternal

grandfather's preteen years adversely affected the health of his grandsons, increasing their relative risk of an early death by about twofold. Surplus food for a paternal grandmother in utero or during infancy adversely affected the health of her granddaughters, to a slightly greater degree.

Given the limits of epidemiological analyses, Richardson and others are concerned that unrecognized factors might have influenced these results, and they wonder if these intergenerational associations could be statistical flukes. Pembrey thinks not, noting that the critical periods revealed in the smoking and food studies coincide with when eggs are maturing in girls and sperm production is about to begin in boys.

Based on his findings, Pembrey speculates that smoking, nutrition, and perhaps other lifestyle factors can cause semipermanent changes in the germ line during these critical periods. Most researchers had thought that such epigenetic changes only occurred while a person was developing in the womb. Pembrey's results also indicate that post-development effects can be transmitted through the paternal line. They're "proof of principle. The sperm have captured information about the ancestral environment, and this is modifying the development and health of subsequent generations," he says.

If so, epigeneticists need to give more thought to what fathers contribute, says James Curley of the University of Cambridge, U.K. "The mechanism underlying [sperm-based transmission] will be a big area in epigenetics," he predicts.

Supplements Restore Gene Function via Methylation

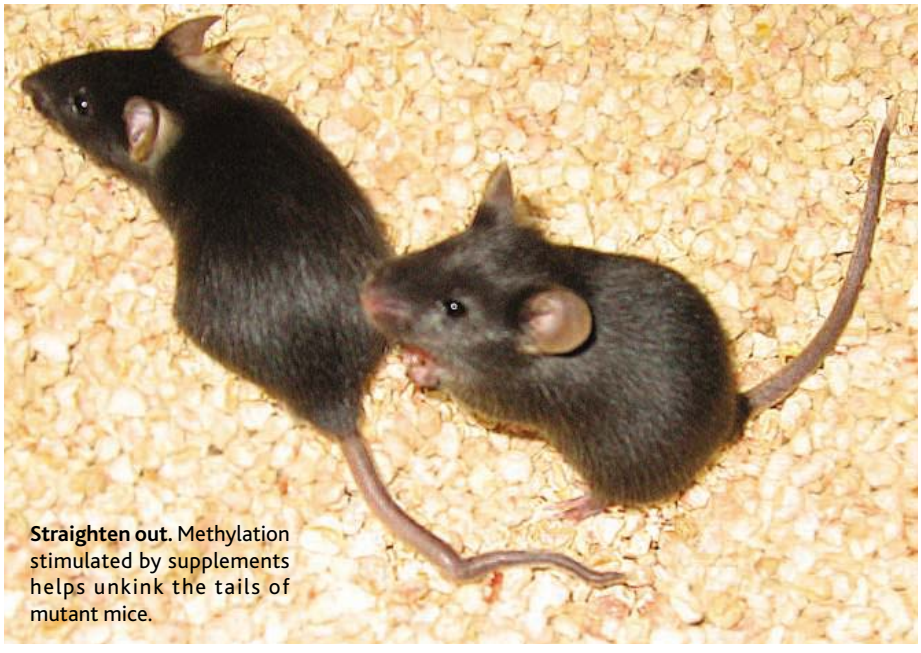
It has long been known that pregnant women who consume insufficient folic acid, a B vitamin, run an increased risk of having babies with spina bifida or similar neural tube defects. Yet biologists are still teasing out exactly what this vitamin does for the developing fetus. At the meeting, Robert Waterland, an epigeneticist at Baylor College of Medicine in Houston, Texas, presented evidence from mice that methylation of DNA—a chemical modification that can shut down genes—can be key.

Folic acid does restore gene function in mutant mice that have improper DNA methylation patterns, the researcher reported. However, Waterland has also found that the supplement-induced changes in DNA methylation might not be all that predictable—they appear to occur at different points in time during embryonic development and to affect only specific tissues, he reported.

These mouse results may have implications for supplement use in both pregnant women and the public at large. "People are taking massive quantities of vitamins, and we don't have any idea what these potential methyl donors are doing," says Adele Murrell, a geneticist at the University of Cambridge, U.K.

Waterland first observed the embryonic impact of folic acid and other methyl donors 2 years ago, while working with Randy Jirtle at Duke University in Durham, North Carolina. At that time, he examined a strain of off-colored mice that has a defect in a pigment gene called *agouti*—the gene is defective because a mobile bit of DNA called a transposable element had inserted itself in some of the nearby DNA that regulates the gene's expression. The transposable element short-circuits methylation of this regulatory region, causing the gene to be overactive. As a result, yellow or mottled coats are common in these animals. But litters born to dams fed supplements of folic acid, a rich source of methyl groups, were primarily the typical brown. Waterland and his colleagues subsequently found that the supplements caused an increase in the density of methyl groups on and around the *agouti* gene, overriding the transposable element's effects.

Waterland has since investigated a gene that may be more relevant to human disease. The *axin* gene helps set up the dorsal-ventral axis in embryos and also requires methylation to work properly. Many mice with an *axin* disrupted by a transposable element embedded in it typically develop mild to tightly angled kinks in their tails. Again, methyl donors can come to the rescue. Waterland reported that receiving folic acid supplements during pregnancy reduced by half kinking in the pups' tails. Taken together, "Waterland's data are the most convincing positive finding with respect to whether diet has any effect on the methylation patterns and expression of a particular [gene]," says Carmen Sapienza, a geneticist at Temple University in Philadelphia, Pennsylvania.



Straighten out. Methylation stimulated by supplements helps unkink the tails of mutant mice.

CREDIT: R. WATERLAND/BAYLOR COLLEGE OF MEDICINE

Folic acid supplementation altered the methylation of the two genes in different ways, however, illustrating the complexity of the phenomenon. In the *agouti* mice, the supplements increased methylation of the gene in a variety of tissues, and the change was most pronounced early in pregnancy. But in the case of the *axin* mice, that gene's methylation remained low early in pregnancy and only increased later on, as the tail formed, Waterland reported. Moreover, the increase occurred only in the tissue giving rise to the tail. These two observations suggest to him that DNA methylation produced by vitamin supplementation can be tissue-specific and, depending on the gene involved, can occur at different times over the course of a pregnancy.

Waterland's research may one day lead to more sophisticated timing of when to give vitamin supplements to pregnant women or anyone else. "If we can understand critical windows and when methylation is beneficial," says Patrick Stover, a nutritional biochemist at Cornell University, "that would totally change the concept of how we set dietary requirements during pregnancy and how we think about preventive medicine."

–ELIZABETH PENNISI

Glowing prospects. Sleek, high-efficiency organic-based lights should be on the market by 2007.

Organic LEDs Look Forward to a Bright, White Future

A new type of light-emitting diode may be set to give light bulbs and fluorescent tubes a run for their wattage

BOSTON—If you want to save the world, you might start by getting rid of the light bulb. In the United States alone, lighting sucks up more than 6 quadrillion BTUs of energy every year, 17% of all the energy used in buildings. Incandescent bulbs turn about 90% of that energy into not light but heat. Fluorescents do better, converting 70% of the energy they use into light. But researchers have spent decades working to create novel semiconductor-based light-emitting diodes (LEDs) that do even better. Red LEDs and other colors made from inorganic compounds are already in widespread use in traffic lights, car taillights, and other niche applications. Inorganic white LEDs are also on the market. But so far, all of them remain too costly for general lighting use. Now a new competitor is coming on strong.

At a recent meeting of the Materials Research Society* here, researchers from Japan, Germany, and the United States reported steady progress in turning thin organic films into high-efficiency lights. Because such films are likely to be made with inexpensive organic starting materials, they are potentially very cheap to manufacture, even in large panels. That day isn't here yet, but with prototype products already in development, the first white organic light-emitting diodes (OLEDs) for general lighting are expected to hit the market in 2007. The efficiency of these new OLEDs "is moving up quite fast," says Stephen Forrest, an OLEDs researcher at Princeton University.

That pace of improvement has recently caught the attention of numerous lighting companies, which are also pushing the technology forward. "No one cared about [white OLEDs] until a few years ago," says Anil Duggal, an OLED researcher at General Electric in Niskayuna, New York. Duggal says most of the interest in OLEDs until now has been for making flat-panel displays for everything from cell phones to wall-sized televisions. That's partly because the display market, which brings in about $100 billion a year worldwide, is twice the size of the lighting market. For displays, OLEDs also had the advantage of being ultrathin, a feature many experts believe will command a premium on the market and compensate for the fact that the early devices had relatively poor efficiency. But to compete in the lighting market, where their sleek appearance isn't as critical, OLEDs had to become both better and cheaper. "You need higher efficiency and brightness for lights, in order for OLEDs to carve out a niche in the market," Duggal says. Now, there is impressive progress on both fronts.

At the meeting, Junji Kido, an OLED expert at Yamagata University in Japan, reported that he and his colleagues have produced white OLEDs with an efficiency of up to 57 lumens per watt (lm/W) of power that's fed into them. That's nearly the efficiency of fluorescent bulbs and almost four times that of incandescent lights, which typically operate at 15 lm/W.

That efficiency is a big step up from the first white OLED, which Kido and colleagues produced in 1993. Like all LEDs, that device was made by sandwiching a light-emitting material between two electrodes. When turned on, positive and negative charges pass from the electrodes and into the light-emitting material, where they combine and give off a photon of light. In Kido's initial OLED, the device contained red, green, and blue light–emitting compounds that together produced white light. But the early devices had problems. Their efficiency was meager, at less than 1 lm/W, they required large voltages to drive charges into the light-emitting materials, and they burned out quickly.

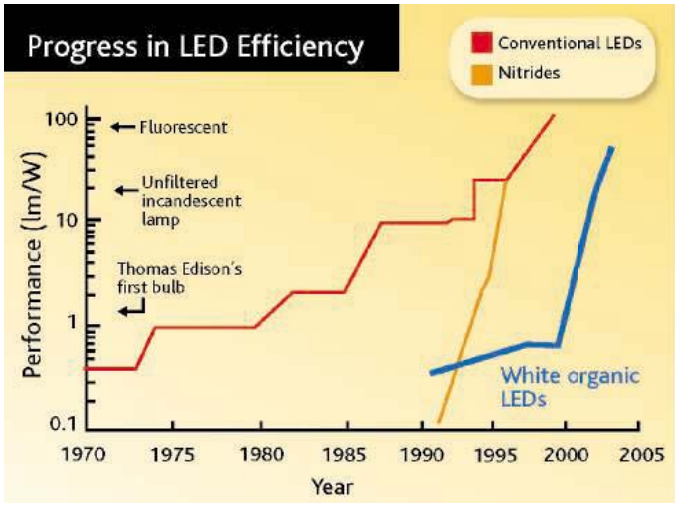
Kido and his colleagues have worked through numerous generations of devices, steadily improving their efficiency, lifetime, and operating characteristics. One of the biggest changes, pioneered by Kido's and Forrest's groups and others, has been in switching from light emitters that fluoresce to ones that are phosphorescent. The change comes in the quantum-mechanical details of how these materials turn electrical charges into light. When negatively charged electrons and positively charged "holes" meet in organic materials, they create electron-hole pairs called excitons that quickly "decay" and give off their energy either as a photon of light or as heat. In addition to carrying charge, electric charges harbor a property known as spin. And because of the precise way in which the spins align in these excitons, 25% of the excitons become what is known as "singlet" excitons, whereas the other 75% become "triplet" excitons. That's important, because fluorescent compounds can convert only singlet excitons into photons

CREDIT: J. KIDO/YAMAGATA UNIVERSITY

* 28 November–2 December.

as the exciton decays. The triplets, meanwhile, just give up their energy as heat. Phosphorescent dyes, however, can convert both singlet and triplet excitons into light, making them potentially much more efficient.

At the meeting, Kido reported that he and his colleagues have synthesized novel phosphorescent compounds and used them to make high-efficiency white OLEDs. Kido's group, for example, reported that they have made a new blue OLED with a record-breaking efficiency of 42 lm/W when it puts out a relatively dim 100 candelas per square meter (cd/m^2) and 31 lm/W at a bright 1000 cd/m^2. At the heart of the blue device was a blue light–emitting phosphorescent compound called Flrpic. One key to improving the device was that the researchers surrounded the Flrpic-containing layer with layers of other compounds that allow triplet excitons to reside there only if they have very high energies. In effect, this creates an energetic well in the light-emitting material, so that once the excitons fall into that region, they can't get back out, raising the likelihood that they will decay in the presence of the blue phosphor and give off blue light. To get white light, the researchers then added a yellow phosphor to their blue light–emitting layer, converting some of the emitted light to yellow, which combined with the blue to give off white. The new results are "fantastic work," says Yang Yang, a physicist at the University of California, Los Angeles (UCLA). And at 57 lm/W, the efficiency, Yang adds, "is a very impressive number."



Stepping up. White OLEDs are now almost as efficient as fluorescent tubes.

Bright idea

It was far from the only impressive number reported at the meeting. Forrest outlined a new strategy for improving OLED efficiency that eventually may surpass even the all-phosphorescent devices. Working with chemist Mark Thompson at the University of Southern California in Los Angeles, Forrest has been reconsidering the trend toward making OLEDs with phosphors only. The reason, he explains, is that for phosphor-based OLEDs to turn out white light, they must first convert singlet excitons to triplets, which then emit the light. "You pay a price for that," as this initial conversion step lowers the overall efficiency of the device.

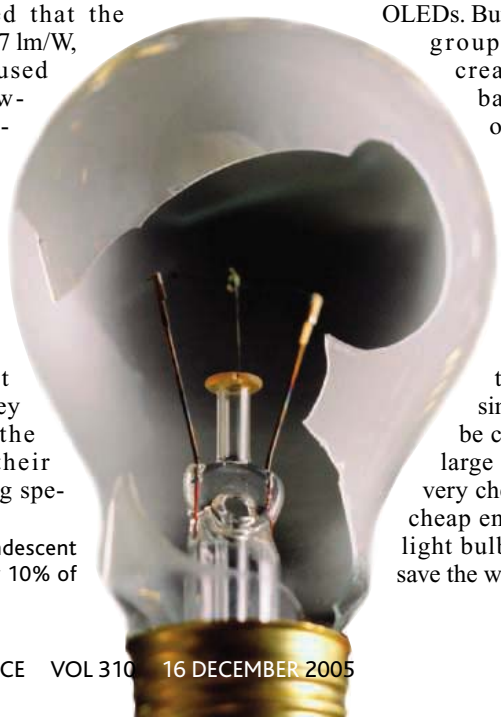
So Forrest and his colleagues have made LEDs with a combination of phosphorescent and fluorescent light emitters. They designed the devices so that the singlet excitons, which have a higher amount of energy than the triplet excitons, were directed to a fluorophore to generate blue light, which requires higher energy charges to make than other colors. The lower energy triplet excitons were then directed to additional layers harboring red and green phosphors. The researchers were able to target them in that way because the singlet excitons are too short-lived to travel far through the device before decaying and giving up their energy. By contrast, triplet excitons are long-lived and can travel comparatively long distances.

Forrest's team placed their blue light–emitting fluorescent dopants in a central region where the excitons initially form and sandwiched them between layers containing the red and green phosphors. Because the triplet excitons couldn't generate blue light in the fluorophores, they passed right through that layer on their way to the phosphorescent-containing layer. Forrest reported that the device achieved 27 lm/W, even though it used a relatively low-efficiency blue-light fluorophore. If the best blue fluorophore were used in its place, Forrest estimates, the efficiency would likely double.

Both Forrest and Kido say they can also boost the efficiency of their OLEDs by adding special coatings to the outside of the glass that sits at the top of OLED devices. Without such a coating, most photons reflect off the glass-air interface and bounce back inside the device, where many of them are reabsorbed and wind up generating heat instead of light. When Kido's group added an antireflective coating to their best white OLEDs, the efficiency climbed from 36 lm/W to 57 lm/W. Forrest says he expects he would get a similar jump. Given the steady progress with white OLEDs, Karl Leo, an OLED expert at the Technical University of Dresden in Germany, says the future looks bright. "With OLED lighting, it should be possible to surpass the fluorescent tube," Leo says. "Whether it will be cheap enough and stable enough is still an issue."

But groups reported progress on those fronts as well. Kido is part of a large Japanese consortium that is creating industrial-scale machines to manufacture large white OLED panels at high speed and low cost. Working with Matsushita and other companies, the group has already produced 30-by-30-centimeter panels and expects to begin selling products within 2 years.

Even with novel machinery, however, conventional OLEDs, which are produced by depositing as many as 10 or more successive layers of materials in a vacuum, could still be relatively expensive to make. But progress is also coming quickly in making much simpler devices with a single polymer layer between electrodes. Such devices typically have far lower efficiencies than the standard OLEDs. But at the meeting, Yang's group at UCLA reported creating white polymer-based OLEDs that put out 14 lm/W, and a group led by Franky So of the German lighting company OSRAM reported reaching 16 lm/W—already as efficient as incandescent light bulbs. And because the devices consist of simple polymers that can be cast from solution over large areas, they look to be very cheap to make—perhaps cheap enough to dethrone the light bulb and do their part to save the world.



Energy hog. Incandescent bulbs convert only 10% of energy into light.

–ROBERT F. SERVICE

SOURCE: ADAPTED FROM S. FORREST/PRINCETON UNIVERSITY AND J. R. SHEATS *ET AL.*, *SCIENCE* **273**, 884 (1996); CREDIT: GETTY IMAGES

Random Samples

Edited by Constance Holden

World's tallest building.

Giving the Earth a Poke

The weight of the world's tallest building may have been great enough to trigger earthquakes.

The 101-story Taipei 101, completed in 2003, rises 509 meters over the capital of Taiwan. The tower's 705,132 tons of steel and concrete also exert considerable pressure on the ground below it. So when magnitude-3.8 and -3.2 quakes struck directly beneath the building in late 2004 and early 2005, seismologist Cheng-Horng Lin of Academia Sinica's Institute of Earth Sciences in Taipei took an interest.

The quakes struck a previously unrecognized fault 10 kilometers down that before construction had produced only minor tremors too small to be felt. The building's weight is applied in just the way needed to make the fault slip as it did in 2004, Lin found. "[T]his megastructure might very well have triggered" the quakes, he concludes in a 30 November *Geophysical Research Letters* paper. The seemingly stressed fault demands close monitoring, he adds.

Human activities such as nuclear explosions and filling water reservoirs have been known to trigger quakes, but a building has never before been fingered. Seismologist Ross Stein of the U.S. Geological Survey in Menlo Park, California, says he's not convinced that Taipei 101—"just a woman's high heel on a slightly larger scale"—could cause quakes. The stresses at the base of the building can be quite high, he notes, but they rapidly decay deeper in the earth because the building is so narrow.

Still Missing the Mark

Although many states have recrafted their public school science standards in the past 5 years, "we're no better off now than before," according to a report issued last week by the Thomas B. Fordham Foundation in Washington, D.C. "Science education in America is under attack, with 'discovery learning' on one flank and the Discovery Institute on the other."

The foundation, which supports research on education reform, graded each state based on the clarity and quality of its standards. Seven states, led by California, got an "A." Virginia was most-improved, rising from a "D" in the foundation's 2000 report to an "A." Of the 13 that received an "F," all except New Hampshire are in the South or West.

Lead author of the report is biologist Paul R. Gross, former head of the Marine Biological Laboratory in Woods Hole, Massachusetts. Gross says the problem with precollege science education is much bigger than the debate over teaching evolution. "Certainly some states do an awful job addressing evolution, but for the most part these states also do an awful job addressing the rest of science," he says. See www.edexcellence.net for *The State of State Science Standards 2005*.

Peruvians Pressure Yale on Artifacts

Peru has joined the throng of nations seeking the return of native archaeological treasures that reside in foreign museums. The Peruvian government is threatening to sue Yale University for failing to return some 5000 artifacts that renowned researcher Hiram Bingham excavated during three visits to Machu Picchu nearly a century ago.

Bingham was the first foreigner to behold the dramatic site high in the Andes, a stronghold of the Incan empire that was abandoned in the early 16th century. He found troves of mummies, pottery, and sculpture, and with the permission of the Peruvian government, the objects were taken to Yale—but they were supposed to be returned within a few years.

Peruvians want the return of items such as this ritual offering vessel.

Negotiations have been going on for several years, but with Peruvian elections coming up next April and the centennial of Machu Picchu's 1911 discovery by outsiders approaching, the government is putting the pressure on. "Machu Picchu is a very, very potent symbol for Peru as well as for all native peoples of the Americas," says Clark Erickson, an archaeologist at the University of Pennsylvania. Foreign Minister Oscar Maurtua told reporters in Lima last week that although he would prefer an out-of-court settlement, he believes Peru could win in a court battle. Yale spokesperson Thomas Conroy says the university hopes to find a resolution through joint exhibitions of the material in Peru as well as in the United States. Many of Machu Picchu's treasures have been on public display for the first time in a Yale traveling exhibit that opened in 2003.

New Species in Borneo?

A mysterious new catlike animal has been glimpsed by cameras set up in the dense central forests of Borneo by researchers from the Swiss World Wildlife Fund (WWF). With dark red fur and a long bushy tail, it may be a type of marten or civet. WWF biologist Stephan Wulffraat said that the animal was unfamiliar to the locals, and wildlife experts were stumped. Researchers hope to nail down its identity by trapping a live one. Although the animal lives in Kayan Mentarang National Park, WWF says plans by the Indonesian government to build a giant palm oil plantation in the area pose a threat to it.

CREDITS (TOP TO BOTTOM): SIMON KWONG/REUTERS; YALE PEABODY MUSEUM; DAVE AUGERI/WWF-INDONESIA

PEOPLE

Edited by Yudhijit Bhattacharjee

AWARDS

Reality TV. Many television shows about science portray it as a purely rational pursuit. A Belgian TV producer has won a top science communication prize for showing the emotional drama of searching for breakthroughs.

Jos Van Hemelrijck's *Overleven*—a Flemish play on words meaning "about life" and "survival"—documents the trials and tribulations of the scientific method by trailing a single researcher every week. "We try to follow a false lead and dramatize the disappointment," he says. Episodes focus on those whose work has an impact on society, such as a stem cell expert and a biologist who trains rats to be minesweepers. Through realistic portrayals of scientific life, Van Hemelrijck says, the series strives to meet the "demand for deeper science."

For his efforts, Van Hemelrijck shares the European Union's $300,000 Descartes Prize for science communication with Bill Bryson, author of the layman's guide to science, *A Short History of Nearly Everything*; Swedish medical doctor Carl Sundberg; Danish astrophysicist Anja Andersen; and Michael Seifert, who started the "Children's University" to stimulate scientific interest in German schoolchildren.

The E.U. has also named five scientific groups as the winners of its $1.2 million Descartes research prize. Details are at www.cordis.lu/descartes.

RISING STARS

Preparing for takeoff. The winners of the Siemens Westinghouse high school competition in math, science, and technology are in. Michael Viscardi (below, right) of San Diego, California, a homeschooled student with a flair for music composition, will

receive the $100,000 grand prize for finding elegant and computer-applicable solutions to a complex mathematical problem. Aspiring actress/biologist Anne Lee, a student at Phoenix Country Day School in Paradise Valley, Arizona, and future computer scientist/intellectual property lawyer Albert Shieh, a student at Chaparral High School in Scottsdale, Arizona, share the $100,000 team prize for developing software to analyze high volumes of genetic data.

ON CAMPUS

Attacked. When religious studies professor Paul Mirecki of the University of Kansas, Lawrence, decided to offer a class on creationism, intelligent design, and "other religious mythologies" last month, he knew that his inbox was going to get flooded with nasty e-mails. What he probably didn't expect was a physical beating, followed by the loss of his title as department chair.

The thrashing took place on 5 December, after Mirecki had already canceled his proposed class. He told police that two men in a pickup truck followed him as he was driving to breakfast in rural Douglas County and pummeled him when he got out of his car. Mirecki drove to a hospital, where he was treated for bruises. He told reporters his assailants referred to the controversy during the attack. Police have yet to make any arrests in the case.

Mirecki has come in for intense criticism from politicians and Christian groups not just for proposing the class but also for describing it in an e-mail as "a nice slap" in the "big fat face" of "fundies," and for many other e-mails containing derogatory remarks about Christians, says university spokesperson Lynn Bretz. On 7 December, at the urging of his colleagues, he stepped down as chair of his department.

The university still hopes to offer Mirecki's proposed course taught by someone else, says Bretz.

JOBS

New blood for CDC. After 2 years of reorganizing and the loss of many senior scientists, the Centers for Disease Control and Prevention in Atlanta, Georgia, last month promoted three staffers to key leadership posts in infectious diseases. Rima Khabbaz will direct the National Center for Infectious Diseases, Anne Schuchat will head the National Immunization Program, and Kevin Fenton will lead the National Center for HIV, STD, and TB Prevention.

POLITICS

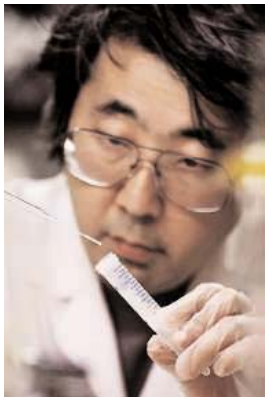
Not in my backyard. You can be too close to a good thing—that's what some residents of Anchorage, Alaska, are saying to a neighbor who wants to put a particle accelerator in his house. Engineer Albert Swank Jr. plans to use the 60-ton, room-sized cyclotron, donated by Johns Hopkins University, to generate radioactive isotopes for a type of medical imaging called positron emission tomography (PET). "I lost my father [to cancer] in 1982," says Swank, 55, "and I decided at that point to start working on bringing a PET facility to Alaska." The state currently has two such scanners, Swank says, but must fly in isotopes from Seattle, Washington. Swank, who built his own cyclotron as a teenager, says he also hopes to inspire local students to pursue science by introducing them to the device.

Some residents claim the machine is a radiation hazard. "We'd be pleased as punch to bring this technology to Alaska," says Allan Tesche, a member of the Anchorage city council, "but it belongs in hospitals and industrial areas." Tesche says that Swank's plans violate zoning laws, and he has introduced an ordinance that would explicitly forbid home cyclotrons. Swank counters that he already has all the permits required to install the machine in January, perhaps in his garage.

CREDITS (TOP TO BOTTOM): UNIVERSITY OF KANSAS; MEL NUDELMAN; ALBERT L. SWANK

Asian Scientists and the "Glass Ceiling"

IN HIS ARTICLE "A GLASS CEILING FOR ASIAN scientists?" (News Focus, 28 Oct., p. 606), J. Mervis raised the interesting question of why Asian scientists, who are abundantly present in U.S. research and industrial labs and academic institutes, do not hold positions and titles as senior leaders. This is strongly supported by available statistics from the National Institutes of Health, the Society for Neuroscience, and the American Society for Biology and Molecular Biology.



Asian scientists are prevalent in U.S. labs but found in much lower numbers in leadership positions.

We applaud these efforts in revealing some of the facts and concerns behind this "glass ceiling." From what we have gathered and what has been shown, Asian-Americans are represented currently in less than 10% of higher decision-making positions in their particular professions. This is true in academia, government, and private enterprise. Although the reasons behind this issue are complicated and certainly need to be dealt with appropriately, we believe it is and will continue to be America's loss for not tapping into such a vastly talented pool for current and future leaders. It is also time for Asian-American scientists to assert themselves and to embrace any challenges from the decision-making hierarchy. Our hope remains that Asian-Americans and non–Asian-Americans will work together to remove barriers for the common good of this great nation. On this note, it is encouraging to see that some of the major institutions in this country have appointed Asian-Americans to prominent leadership positions; for example, two of the seven chairs in the Basic Science Research Departments at the M.D. Anderson Cancer Center are held by Asian-Americans.

MIEN-CHIE HUNG,[1] KENNETH FONG,[2] JOSEPH K.-K. LI[3]*

[1]President of the Society of Chinese Bioscientists in America (SCBA) and Professor and Chair, Department of Molecular and Cellular Oncology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA. [2]President-elect of the SCBA and Chairman, Kenson Venture, LLC, Palo Alto, CA 94301, USA. [3]Executive Director of the SCBA and Professor, Department of Biology, Utah State University, Logan, UT 84322–5305, USA.
*To whom correspondence should be addressed. E-mail: josephli@biology.usu.edu

WE READ THE NEWS FOCUS ARTICLE "A GLASS ceiling for Asian scientists?" (J. Mervis, 28 Oct., p. 606) with chagrin and numbers envy. Latino/Hispanic scientists at the National Institutes of Health (NIH) are essentially an endangered species, despite a 10-year effort by Hispanic scientists to have the NIH address the disparity (see http://heo.nih.gov/). In contrast to Asian scientists, in April 2005 Latinos made up 5.3% of tenure-track investigators versus 4.5% in 1994 (21.5% and 10.2%, respectively, for Asians) and 2.5% of senior investigators (1.5% in 1994) (9.2% and 7.2%, respectively, for Asians) (*1*). In fact, Latinos account for 4.3% of medical officers, 3.4% of biologists, and 2.5% of chemists in the NIH workforce—significantly below the figure for the U.S. Civilian Labor Force (*2*). Hence, there seems to be little opportunity for Latinos to become the "right-hand men" (or women) noted by Dr. Jeang in the article.

RAYMOND MEJIA,* OFELIA OLIVERO,† MIGDALIA RIVERA-GOBA,‡ ANA ANDERS,* CARLOS CABAN,* MARTA LEON-MONZON,* ERNEST MARQUEZ*

National Institutes of Health, Bethesda, MD 20892, USA.
*Past presidents of the NIH Hispanic Employee Organization (NIH-HEO).
†Current president of the NIH-HEO.
‡President-elect of the NIH-HEO.

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1. See http://heo.nih.gov/files/Tenure_Track_and_Tenured_Scientists.ppt.
2. See http://heo.nih.gov/files/NIH_PROFILE_BY_JOB_SERIES.ppt.

THE NEWS FOCUS ARTICLE "A GLASS CEILING for Asian scientists?" by J. Mervis (28 Oct., p. 606) attracted my attention. When you look at these numbers, on the surface, one may draw the conclusion that Asian scientists are underrepresented. However, it should be taken into consideration that many Chinese scientists have been in the United States for less than 20 years, since China's "Open Door" policy. With graduate study, postdoctoral training, and junior faculty positions, a scientist may need more than 15 years to build up her or his experience and reputation to be a leader in research laboratories and in scientific societies. The visibility of Asian scientists in scientific leadership should increase with time.

DIANHUA JIANG

Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520–8057, USA. E-mail: dianhua.jiang@yale.edu

I WAS APPALLED WHEN I READ ABOUT THE percentage of Asians in tenure-track positions at the National Institutes of Health (NIH) in the News Focus article "A glass ceiling for Asian scientists?" (J. Mervis, 28 Oct., p. 606). According to the 2000 Census, Asians make up only 3.6% of the population of the United States, yet 21.5% of researchers at NIH are Asian. Could this be due to a hiring bias on the part of NIH? I'm not Asian so apparently I do not have the inherent qualities to make a good researcher and have had to forge out on my own to conduct science. Bigotry at workplaces like NIH will only harm our country in the long term, and I hope that articles like this, rather than postulating some fanciful glass ceiling, will shed light on the immoral and unfair hiring practices that go on currently at our public institutions.

JEFFREY B. STEWART

Aeri Park Institute of Molecular Biology and its Applications (APIMBA), 3000 Kent Avenue, West Lafayette, IN 47906, USA.

I WRITE TO ADDRESS COMMENTS ATTRIB-uted to me in the News Focus article "A glass ceiling for Asian scientists?" (J. Mervis, 28 Oct., p. 606). Some comments cited in the article were from an e-mail and referred to trainees and the difficulties they, particularly those who are foreign nationals, face in making the transition to positions as independent scientists. For example, my comments indicated that trainees were "not in a position" to serve on the committees of scientific societies because they are at a very early stage of their careers. I have spent the better part of my academic career working to increase the representation of women scientists at senior levels. Having actively advocated for equity in the promotion and participation of women scientists, I am hardly in favor of bias against other groups.

Scientific societies are not involved in the hiring or promotion of their members and thus do not control the numbers of underrepresented minorities who advance to the faculty ranks from which society committee members are drawn. Scientific societies can, however, ensure that the accomplishments of all their members are

recognized through presentations at society-sponsored meetings as well as service on society committees. They can also take a leadership role in ensuring that the issue of equity remains a priority within the scientific community as a whole. I believe that this article has raised the awareness of all of us to this problem and hope that it will help to achieve equity for all members of the scientific community.

LINDA J. PIKE
Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, 660 South Euclid, Box 8231, St. Louis, MO 63110, USA.

How to Cut World Hunger in Half

THE CO-CHAIRS OF THE UN TASK FORCE ON Hunger, created in 2002 to determine how to meet the UN hunger Millennium Development Goal (*1*, *2*), recently summarized their findings in *Science* ("Cutting world hunger in half," P. A. Sanchez and M. S. Swaminathan, Policy Forum, 21 Jan., p. 357).

Roughly 80% of the world's hungry live in rural areas of the least developed countries and have no power in society. These people are mainly farmers and need incentives to invest in their land to intensify agricultural production (*3*, *4*). However, urban, mostly well-educated people, who do not depend on agriculture for their survival, frequently determine agricultural policies (*5*–*7*).

The Task Force on Hunger report does not make its analysis from the interests of the poorest, rural people, nor does it assess how farmers would get necessary incentives (such as better prices) for their agricultural products (*8*, *9*). According to the UN Development Programme's *Human Development Report,* "The basic problem to be addressed in the WTO [World Trade Organization] negotiations on agriculture can be summarized in three words: rich country subsidies" [(*10*), p. 10]. Wealthy countries give 1 billion U.S. dollars per year in agricultural aid to developing countries, while they subsidize their own agriculture with nearly 1 billion U.S. dollars per day [(*10*), p. 130].

According to the *Human Development Report* "Rich country consumers and taxpayers are locked into financing policies that are destroying livelihoods in some of the world's poorest countries… WTO rules threaten to systematically reinforce the disadvantages faced by developing countries and to further skew the benefits of global integration towards developed countries… The unbalanced agenda pursued by rich countries and failure to tackle agricultural subsidies are at the core of the problem" [(*10*), p. 10]. UN Secretary General Kofi Annan has said that "The [Millennium Development Goals] can be met by 2015—but only if all involved break with business as usual and dramatically accelerate and scale up action now" [(*10*), p. 5].

To avoid that disaster, wealthy countries must accept free trade principles. At the WTO meeting in Hong Kong in December 2005, they must agree to (i) make deep cuts in wealthy countries' support for domestic agriculture and prohibit export subsidies and (ii) make deep cuts in barriers to exports from developing countries (*10*).

According to Kirkpatrick and George, trade liberalization "can contribute positively to the [Millennium Development] goal of eradicating extreme poverty and hunger" [(*11*), p. 3]. Hoekman *et al.* write that a 50% reduction "in border protection will have a much larger positive impact on developing economies' exports and welfare than a 50% reduction in agricultural subsidies" [(*12*), p. 175]. Price incentives through cancelled trade barriers and subsidies (*13*, *14*), as well as a redressed rural-urban balance in living conditions and production (*7*), are therefore necessary but not sufficient to halve the number of hungry by 2015. In contrast to the effects of aid and debt cancellation, the money from higher agricultural prices goes directly into the pockets of the poorest people and of the farmers in poor countries.

"In contrast to the effects of aid and debt cancellation, the money from higher agricultural prices goes directly into the pockets of the poorest people...."
–LINDSKOG

PER LINDSKOG
Department of Science and Technology, Linköping University, S-601 74 Norrköping, Sweden. E-mail: Per.Lindskog@itn.liu.se

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Cognitive Unbinding in Sleep and Anesthesia

I READ WITH GREAT INTEREST THE REPORT "Breakdown of cortical effective connectivity during sleep" by M. Massimini *et al.* (30 Sept., p. 2228). As in the field of sleep research, the traditional view in anesthesiology has been that the brain is somehow "shut off" under general anesthesia. More recent data and formulations of anesthetic mechanism suggest, however, that it is not the neural activity but rather the integration of neural information that is inhibited.

It has been previously reported that numerous general anesthetic agents from various pharmacologic classes induce a similar pattern of functional uncoupling between the cerebral hemispheres, as well as between the rostral and caudal poles of the brain (*1*). These and other data describing anesthetic-induced interruption of cognitive binding processes have given rise to the theory of general anesthesia as a "cognitive unbinding" (*2*, *3*). Recent data from magnetic resonance imaging support this by demonstrating loss of functional cortical connectivity under sevoflurane anesthesia (*4*).

The integration of cognitive information as an essential feature of consciousness has its philosophical origins in the 18th-century epistemology of Kant and has made its modern neuroscientific appearance as the cognitive binding problem and the information integration theory of Tononi (*5*). Massimini *et al.* suggest that

the loss of integration during sleep supports its central role in consciousness, a claim that is strengthened by current perspectives of the general anesthetic mechanism. Furthermore, I would suggest that the loss of cortical effective connectivity, or "cognitive unbinding," may be the common feature of general anesthesia and sleep that has long been hypothesized but never identified.

GEORGE A. MASHOUR
Anesthesia and Critical Care, Massachusetts General Hospital, Clinics 309, 55 Fruit Street, Boston, MA 02138, USA.

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Response

MASHOUR'S COMMENTS GO RIGHT TO THE point. According to the information integration theory (*1*), the neural substrate of human consciousness is a complex of neuronal groups, largely contained within the thalamocortical system, that has two key properties: (i) it behaves as a single, integrated entity and (ii) it has a large repertoire of different states or firing patterns. Such a combination of properties requires that distributed neuronal groups that are functionally specialized can interact effectively through multiple pathways.

If the critical interactions among such specialized neuronal groups are compromised, consciousness should fade, even if neural activity is preserved. Our Report indicates that this is precisely what happens during slow wave sleep early in the night. Mashour and others have pointed out that a similar situation may occur under anesthesia (*2*–*4*). For example, recent neuroimaging studies have revealed that functional connectivity (correlated activity) between distant cortical areas and between thalamus and cortex is reduced by different anesthetic agents (*5*).

An important task for the future will be to determine whether effective connectivity (the ability of one cortical area to causally affect other areas) is also reduced by anesthetic action. It would be important to know whether a reduction of effective connectivity also underlies the anesthetic action of ketamine and other substances that do not reduce cortical metabolism (*4*). It would also be important to establish whether a breakdown in cortical effective connectivity is due primarily to a direct action by anesthetics on cortico-cortical connections, or if it results indirectly from a depression of cortico-thalamic and thalamo-cortical interactions. Although many anesthetics have a disproportionate effect on thalamic metabolism or blood flow (*6*), this may be an inevitable consequence of a diffuse reduction in cortical input to the thalamus (*4*). Finally, it would be valuable to determine whether the transition between just-conscious to unconscious that often occurs with slight changes in anesthetic concentration is predicted more reliably by changes in effective connectivity than by changes in overall metabolism or neuronal activity (*4*).

GIULIO TONONI AND MARCELLO MASSIMINI
Department of Psychiatry, University of Wisconsin, Madison, 6001 Research Park Boulevard, Madison, WI 53719, USA.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

CORRECTIONS AND CLARIFICATIONS

Letters: "Response to 'Problems of studying extinction risks'" by M. Cardillo *et al.* (25 Nov., p. 1277). In the third sentence of the last paragraph, "Despite the case for using PICs in extinction risk studies having recently been clearly and elegantly made (*6*)...," the reference citation is incorrect. It should be reference (*7*): D. O. Fisher, I. P. F. Owens, *Trends Ecol. Evol.* **19**, 391 (2004).

Reports: "Patient-specific embryonic stem cells derived from human SCNT blastocysts" by W. S. Hwang *et al.* (17 June, p. 1777). There were errors in Table 2. The corrected table appears at right.

Reports: "Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst" by W. S. Hwang *et al.* (12 Mar. 2004, p. 1669). Contrary to the statements in the second paragraph of text and first paragraph of the supporting online material, which indicated that there was no financial payment to oocyte and cumulus cell donors, some oocyte donors were financially compensated for their donation with a payment of approximately U.S. $1,400.

NT-hESC	Isolation	Plurip	Differ	Pass #	DNA	HLA
–2	ZF - Blast	√	√	P40	Identical	Match
–3	Blast	√	√	P35	Identical	Match
–4, –5	Im mS	√	√, EB	P26	Identical	Match
–6, –7	Im mS	√	EB	P25	Identical	Match
–8	Blast	√	EB	P21	Identical	Match
–9	ZF - Blast	√	EB	P20	Identical	Match
–10	Im mS	√	EB	P19	Identical	Match
–11	ZF - Blast	√	EB	P19	Identical	Match
–12	ZF - Blast	TBD	TBD	P7	Identical	Match

Table 2. Summary of patient-specific human NT-ESC lines. ZF-blast, zona-free blasotcyst; ImmS, immunosurgery; Plurip, Pluripotent; TBD, to be determined; EB, embryoid body; ✓, pluripotency demonstrated by both EBs and teratomas. Normal karyotypes have been shown for each line (female, pink; male, blue).

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Characterization of Excess Electrons in Water-Cluster Anions by Quantum Simulations"

J. R. R. Verlet, A. E. Bragg, A. Kammrath, O. Cheshnovsky, D. M. Neumark

The conclusion by Turi *et al.* (Reports, 5 August 2005, p. 914) that all experimental spectral and energetic data on water-cluster anions point toward surface-bound electrons is overstated. Comparison of experimental vertical detachment energies with their calculated values for $(H_2O)_n^-$ clusters with surface-bound and internalized electrons supports previous arguments that both types of clusters exist.

Full text at www.sciencemag.org/cgi/content/full/310/5755/1769b

RESPONSE TO COMMENT ON "Characterization of Excess Electrons in Water-Cluster Anions by Quantum Simulations"

László Turi, Wen-Shyan Sheu, Peter J. Rossky

We reiterate that the conclusions of our original report are based on identifiable characteristic trends in several observables with cluster size. The numerical comparison between simulated and experimental vertical detachment energies emphasized by Verlet *et al.* reflects quantitative limitations of our atomistic model, but, in our opinion, does not undermine these conclusions.

Full text at www.sciencemag.org/cgi/content/full/310/5755/1769c

Two-Dimensional Science

Jay A. Labinger

French philosopher Jean Baudrillard has given us the simulacrum as one of the defining characteristics of (post)modern society. A simulacrum is a representation that has completely displaced the original it is meant to represent; it has come to seem much more real than its underlying reality (*1*). Christopher Frayling's *Mad, Bad and Dangerous?*—according to the author, the first full-length book to tackle the portrayal of the scientist in popular culture—neatly reflects Baudrillard's conception. Frayling argues that the current popular image of science and the scientist is almost entirely defined by the cinema and other mass media. The power of cinematic images to penetrate the collective psyche, coupled with the fact that scientists have generally been unwilling and/or unable to compete with convincing narratives of their own (culture, like nature, abhors a vacuum), has brought us to the point where "the public's view of science is shaped more by film and television and newspaper headlines than by anything else."

Mad, Bad and Dangerous?
The Scientist and the Cinema
by Christopher Frayling
Reaktion, London, 2005. 239 pp. $35, £19.95. ISBN 1-86189-255-1.

Of course, the relation between the cinema and science is not a new topic: it has provided material for a vast number of essays and books over the years (*2*). But Frayling's take is a little different. Based on his examination of science-themed movies, from *Metropolis* to *The Matrix*, along with the strong similarities in how schoolchildren describe and portray scientists in surveys carried out from 1957 to 2003, he claims that iconic images of the scientist in cinema have become "part of the cultural drinking water." In particular, key features have survived more or less unchanged. Even though the dominant paradigm of the cinematic scientist has evolved considerably through the 20th century, the same conventional stereotypes are found in a wide variety of genres. From tales of mad scientists such as Henry Frankenstein and his successors, to the hagiographic "bio-pics" of famous scientists (such as Louis Pasteur, Paul Ehrlich, Alexander Graham Bell, Thomas Edison, and Marie Curie) popular in the late 1930s and 1940s, the scientist is shown as a misfit, single-mindedly focused on his (sometimes, but rarely, her) work, and isolated from society in general and from the scientific establishment in particular. As Frayling concludes, "[T]he mad scientist and the saintly one are in some ways two sides of the same Hollywood coin."

Frayling's basic contention, that cinematic images have remarkable staying power, certainly rings true. Anyone would instantly recognize Boris Karloff as Frankenstein's monster from the 1931 James Whale version. Another of his examples will be familiar to readers of a certain generation: the illustration of a nuclear chain reaction by way of a table covered with ping pong ball–loaded mousetraps in Walt Disney's *Our Friend the Atom*, which I haven't seen for nearly 50 years but still remember vividly. On a more detailed level, his arguments might have been made a little more convincing. The thematic organization of his film survey sometimes seems arbitrary and forced; also he goes a little too far in trying to separate popular from literary culture. After all, many of the films he considers have origins in "highbrow" literature. Indeed, the very title of the book has neither scientific nor cinematic ancestry—"mad, bad and dangerous to know" comes from a description of Byron by one of his (female) acquaintances.

The archetypal mad scientist. Dr. Henry Frankenstein (Colin Clive) and his assistant Fritz (Dwight Frye) prepare to bring the monster to life in James Whale's 1931 film.

CREDIT: BETTMANN/CORBIS

I have a couple more quibbles: There is very little real science in the book, and what is presented is often a little bit off. (For example, the author claims that Einstein's 1905 paper on special relativity was experimentally confirmed in 1919, but Arthur Eddington's 1919 solar eclipse observations were taken as confirmation of the theory of general relativity; similarly, the main components of the Strategic Defense Initiative are identified as "heat-seeking lasers.") Furthermore, the generally lively and entertaining writing style is periodically marred by interminable run-on sentences that cry out for the intervention of a more assertive copy editor.

But these are not major shortcomings, because Frayling gives us valuable insights about a very real problem. He also offers suggestions for corrective action, although he does not appear to be very sanguine about the likelihood of success. As he repeatedly points out, positive and/or realistic portrayals of scientific practice may be hard to reconcile with the demands of effective dramatic representation. He cites an early example, H. G. Wells's *Things to Come* (1936). Produced as deliberate counterpoint to the dystopian *Metropolis* (1927), this utopian futuristic movie was, unlike Fritz Lang's classic, a total flop. On the other hand, Frayling has not paid much attention to the recent diversification and fragmentation of popular culture. As public reliance on mainstream cinema and network television is increasingly supplanted by hundreds of satellite and cable channels and the Internet, new opportunities for loosening the decades-long hold of the stereotypical scientific image might well open up.

Lastly, I would carry Frayling's concerns even further on one point. If he is correct that the representation of the scientist as anti-establishment outsider is deeply embedded in public opinion, might not that perception contribute to explaining why scientists who adopt heterodox positions—in arenas ranging from global warming to intelligent design—seem to command so much attention in the United States (*3*)? Frayling (who is English) draws no such conclusion; on the contrary, he quotes one commentator: "All these debates about 'creation science' versus 'Darwin' are almost beside the point. The real creation myth of modern times is not Darwin, not Genesis; it is *Frankenstein*." Maybe so, but on this side

The reviewer is at the Beckman Institute, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, USA. E-mail: jal@its.caltech.edu

of the Atlantic, it certainly doesn't look that way right now.

References and Notes

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2. For example, the Society for Literature, Science, and the Arts, an organization focused on the social and cultural dimensions of science and technology, invariably has several sessions on science and film at its annual conferences. Recent programs can be found at www.litsci.org.
3. See C. Mooney, *The Republican War on Science* (Basic, New York, 2005). Reviewed by N. Oreskes, *Science* **309**, 56 (7 October 2005).

10.1126/science.1121899

ENVIRONMENT

Learning to Say "Enough"

Norman Myers

We live in societies where there is never enough and never too much. At the same time we hear endlessly about our overuse of environmental resources, and there is an emerging consensus that we need to do something. Alas, we hear little in pragmatic, everyday terms about what that "something" could be. Instead, we hear vague admonitions to buy green, to be less greedy, and to think long term, among other well-intended practices. Sometimes these exhortations extend to appeals to political leaders to lead by, for instance, making prices reflect all externality costs.

The Logic of Sufficiency
by Thomas Princen

MIT Press, Cambridge, MA, 2005. 417 pp. $72, £46.95. ISBN 0-262-16232-6. Paper, $29, £18.95. ISBN 0-262-66190-X.

Plainly, such simple urgings are not getting us very far. We need to learn more about the hows of changing people's behavior and then formulating a changed-consumption world. In short, we need to gain a better understanding of the kinds of social organization that will lead us toward the promised land of sustainability. In turn, this means developing new principles to reflect the radical changes ahead.

Such is the message of Tom Princen's *The Logic of Sufficiency*, an admirable and timely book. Princen, a sociologist at the University of Michigan, has long pondered the norms of sustainable consumption, especially when grounded in moderation, restraint, and thrift. He postulates a principle of consumption sufficiency, which he believes can reach beyond the oft-urged goal of resource efficiency. Efficient consumption of resources is still consumption. If 100-miles-per-gallon cars enable consumers to save sufficient money to buy more of this and that, the efficiency increases consumption and only postpones the day when we consume less while enjoying greater material well-being. To paraphrase Al Gore, we need life-styles that are not just better off but better.

Princen starts by reviewing the concept of sufficiency, especially the imperative of sufficiency in an ecologically constrained world. After surveying the "brief and curious history" of the term efficiency, he devotes an entire chapter to the issue of efficiency ratios. The book's first half concludes with a critique of activities undertaken to foster greater consumption through increases in both worker productivity and individual spending. The latter point prompts some revisionist thinking; for instance, when a person has a job he enjoys both work and leisure, but if he becomes unemployed, does he then have endless leisure or no leisure at all?

The second half of the book, "Sufficiency on the Ground," examines key questions through specific examples. The Pacific Lumber Company in California could have logged redwoods in perpetuity had it settled for reduced profits today, but adverse discount rates (among other institutional deficiencies) won out over sustainable profits tomorrow. Conversely, a lobster fishery in Maine provides a success story; co-management shared by local lobstermen and state authority has surmounted problems of common-property rights. Toronto Island has achieved what many would view as laughably impossible: a carless community. In all three instances, an "enough" limitation has been paramount—albeit overruled in the first case while winning out in the other two. Also in each instance, the enough limitation reflects both social values and ecological restraints.

The book ends with an assessment of the fundamental question: How much sufficiency do we need to attain ecological stability? Princen postulates that the ultimate arbiter of what constitutes enough has been the institutional framework: "To say enough when more is possible, well, that is irrational. To say too much when life is full of uncertainty is to deny the role of risk taking and exploration and innovation, indeed, human progress." Tradition asserts that there's nothing that can't be made or done bigger or faster or cheaper. Well, there is: Earth's ecological bounds will proclaim "Thus far and no further."

However idealistic Princen's prescriptions may appear to some eyes, he stresses that they are grounded in "established understandings of human capacity." He rejects "prevailing assumptions about humans' inherent short-term thinking, about their inability to self-organize for restrained resource use, about the insatiability of their consumption, about their inability to do much more than work and spend."

If I have a reservation about Princen's views, it is that he seems unduly critical of a strategy that offers vast (though far from all-encompassing) scope for sustainable consumption, namely, efficiency of resource use. Princen rejects that as somehow opposed to sufficiency, yet the two should surely be complementary. Although the reader encounters the efficiency issue at dozens of points in the book, I would like to have seen more on efficiency gurus such as Amory Lovins, Paul Hawken, and William McDonough.

Restraining the catch. A Monhegan Island lobsterman tosses back a healthy lobster.

CREDIT: JEFFREY L. ROTMAN/CORBIS

All in all, *The Logic of Sufficiency* is a first-rate effort at breaking new ground in the consumption debate. It often flies in the face of conventional wisdom—and not only of those who still rejoice in the prospect of endless growth of the established economy (and hence of consumption, usually two-thirds of that economy). Princen also contests the idea that "greening" of economies and consumption will accomplish the sustainability trick; he even sees greening as a distraction from the ultimate strategy of enough-ism. Conversely, he presents his message in strictly pragmatic terms: not as a visionary ideal but as a practical proposition for the Monday-morning world. He is not only a conceptual optimist, he has sufficient faith in human nature to assert that optimal-scale consumption is both a worthy purpose and eminently doable. In any case, although it may initially be difficult to live with sustainable consumption, it will be far more challenging to live by the credo that there can never be any such thing as enough.

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The reviewer is the coauthor, with Jennifer Kent, of *The New Consumers: The Influence of Affluence on the Environment*. E-mail: myers1n@aol.com

RESEARCH FUNDING

Science Foundations: A Novelty in Russian Science

Irina Dezhina and Loren R. Graham

Derek Bok, former president of Harvard University, wrote in 1996 that, of all the institutions in the United States, private foundations "are surely among the least accountable." And he defended that status, saying that, if foundations answered to "outside groups," they might be "more timid and reluctant to back controversial people or bold undertakings." (*1*). In the United States, government foundations such as the National Science Foundation (NSF) have been given a degree of autonomy unusual for government agencies.

The Soviet Union was supposed to be a planned, directed society. Autonomous institutions such as foundations were considered inefficient, politically threatening, and impermissible. Science was promoted generously by the Soviet state, but on the basis of block-funding to institutions, not peer-reviewed grants to individual researchers.

The fall of the Soviet Union and the attempted creation of democracy and a free economy has led, therefore, to a great debate in Russia over how science should be organized and funded (*2*). One of the results of that debate has been the creation of both government and private Russian foundations financing science.

History

The most active foundations in Russia in the first years after the collapse of the USSR were foreign ones. George Soros alone, through his International Science Foundation, provided about $130 million between 1993 and 1996 to support basic research in the natural sciences (*3*). This was "emergency aid" at a time when Russian scientists were poverty-stricken, and Soros' assistance was much appreciated in the scientific community, although criticized occasionally by nationalists who resented foreign involvement in Russian affairs. Although Soros is no longer supporting Russian natural science, other foreign organizations have continued (*4*).

The creation in 1992 of the first Russian government science foundation—the Russian Foundation for Basic Research (RFBR)—brought a profound change in the support of scientific research. This change was more in principle than in fact, because the budgetary funds available to the RFBR have been modest, as shown below. Although much of Russian science continues to be funded as before, from the top down, the creation of the RFBR brought to Russian science a dramatically different conception of how science should be governed and a new method for the financing and management of research projects. The new conception put much more emphasis on individual researchers and less on administrators, and the new method promoted peer review and transparency of financing.

During the organization of the RFBR, Russian officials studied foreign models of science foundations, especially the NSF in the United States (*5, 6*). In addition, they visited the U.S. National Institutes of Health. The Russians were also interested in the Deutsche Forschungsgemeinschaft, or DFG, in Germany (*7*). The most important feature the Russians took from these foreign models was the peer-review process, a new idea for Russia. These foreign foundations were used as examples that could be modified according to local customs and preferences. Unlike the NSF, for example, the RFBR does not have program officers who frequently rotate from universities or other research organizations, and the RFBR also does not engage in science policy endeavors like the NSF's widely circulated "Science Indicators."

Since in Russian the term "science" (*nauka*) includes both the natural and social sciences, as well as the humanities, at first it was thought that one foundation could handle all knowledge. However, natural scientists were usually recruited to work as RFBR officials, and it soon became clear that they favored their own fields, to the detriment of the social sciences and humanities. Furthermore, the latter fields in Russia still suffered from the years of ideological deformation in Soviet times and were in need of special attention. Therefore, a separate Russian Foundation for the Humanities (RFH) was established in 1994. Although the fields it supports (the humanities and social sciences) differ from the RFBR's, it operates on the basis of similar mechanisms and regulations.

Operation and Emphases of the New Foundations

The amount of money that the new foundations give out is, at least by American standards, pitifully small. For research projects today the average grant size is about U.S.$7000 per year for a group of 5 to 10 researchers. The foundations have obviously made the choice to support "more with less" rather than "fewer with more." Average salaries are also low, about $300 per month (including financing from the science foundations and other programs).

FEDERAL ALLOCATIONS TO SCIENCE FOUNDATIONS

	Percent of total government allocations for civilian science			
Foundation	2000	2001	2002	2003
RFBR	5.75	5.8	5.8	4.9
RFH	0.95	0.96	0.96	0.8

Allocations from the federal budget to Russian science foundations. By law, the government obligation was 6.0 and 1.0% for RFBR and RFH, respectively, which was not met for the years shown.

The budgets of the RFBR and the RFH are, according to current legislation, a fixed share (7%) of the total government expenditures on civilian science (*8*) [not counting space science, 40,239.7 million rubles in 2003 (*9, 10*); see table, above]. The inadequacy of this share is illustrated by noting that, in the United States, the NSF traditionally receives about 20% of total federal government expenditures on basic research at academic institutions (and, in the United States, there are many other sources of researcher funding). But the situation is much worse than these statistics indicate, because often the RFBR does not actually receive the funds stipulated in the legislation. In the first 6 months of 1998, for example, the RFBR received less than one-sixth of its designated budget (*11*). With such radical swings in funding, long-range planning and

I. Dezhina is a leading researcher at the Institute for the Economy in Transition, Gazetny per., 5, Moscow 125993, Russia; e-mail: degina@iet.ru. L. R. Graham is a professor of History of Science at Massachusetts Institute of Technology, Cambridge, MA 02139, USA; e-mail: lrg@mit.edu

steady funding of projects were obviously impossible. Delays in funding grants were frequent, often 5 or 6 months (*12*).

Despite their small budgets, the foundations play a very large role in the hopes and aspirations of Russian scientists for the futures of their fields. Opinion surveys of Russian scientists show that the idea of financing science by grants, after initial suspicion, has found broad support. In 1993–94, according to surveys, competitive grant-financing was positively evaluated by 75% of scientists; just a year or two later, in 1995, that number had grown to 86% (*13*). In the summer and fall of 2003, in a postal survey of scientific workers in 10 regions of Russia conducted by one of the authors, the grant system of financing was evaluated positively by 92.9% of male researchers and 80.3% of female researchers (*14*). (The gender difference triggers questions about the role of women in Russian science and possible gender discrimination.)

> "The foundations play a very large role in the hopes and aspirations of Russian scientists."

Yet despite these positive comments about the grant system in principle, the surveys show that Russian scientists are often less pleased with the specific science foundations currently providing grants. One of the chief complaints is that the foundations favor certain scientists in Moscow and St. Petersburg; consequently, negative evaluations grow as one moves away from these cities. A common criticism is that "cliques" of scientists in the two major cities have formed around the foundations, which resist outsiders (*15*).

Russian scientists often remark that foundations support "normal science" but not "competing paradigms" and that it is impossible to make a scientific breakthrough by means of grant financing.

Private Philanthropy

In the past 6 years, something entirely new has appeared in Russia: private philanthropies supporting science and education. It is true that a century ago, in the tsarist period, there were a few organizations similar to private foundations, but nothing of the sort existed in the Soviet and immediate post-Soviet years. These foundations are still very small in terms of the amounts of money they distribute, and their legal and tax positions are still uncertain, but they represent a potentially promising phenomenon (*16*).

The new private foundations support a range of endeavors and scientists. For example, the Vladimir Potanin Foundation provides stipends and grants to students and young teachers doing research. The Foundation for the Assistance to Domestic Science makes grants to researchers within the Russian Academy of Sciences. The Dynasty Foundation helps students and young scientists (up to age 35) in the field of theoretical physics. The Alferov Foundation supports primarily higher education in the natural sciences with stipends and an annual prize to a young scientist. The Scientific Potential Foundation gives grants in the areas of energy, economics, and the computer information technologies.

The total number of grants given by these foundations is less than 2000 annually, and most grants are small, usually under $100 a month, with some grants to particular senior grantees much larger. The sources of these funds are heterogeneous, ranging from individual wealthy entrepreneurs, to industrial companies, to the presidium of the Russian Academy of Sciences, to small donors, to local government officials.

Looking Toward the Future

Although peer-reviewed applications are likely to result in the support of better-quality research, some scientists are unsuccessful and slide back into poverty or leave for other lines of work. The total number of Russian scientists today is less than half of what it was in Soviet times. Adjusting to such Darwinian principles has not only been painful, but even contradicted much of the spirit of "collectivism" still strong in Russian life. However, adjustment has been occurring.

The questions that have emerged among Russian science administrators and scientists as the most important today are somewhat different from those asked only a few years ago. The most pressing questions in the early '90s were "Can Russian science survive?" and "How should scarce resources during a crisis be distributed?" [For a discussion of the general funding situation of Russian science, see (*17*, *18*).] The most pressing current questions are "What is the correct balance between foundation-funding and traditional block-funding?" and "How much independence from government direction should the foundations have?"

Russian science foundations have two serious weaknesses: (i) chronic underfunding which lessens their effectiveness and threatens the hopes of Russian scientists for transparent and noncorrupt financing, and (ii) legal and tax uncertainties which endanger the future and significance of the foundations. As this article goes to press, legislation is pending in the Russian Duma (legislature) that would, if passed, reduce the independence of nongovernmental organizations, both Russian and foreign (*19*). Government foundations, such as the RFBR, do not appear to be threatened, but the current centralizing tendency of the Putin government undermines autonomy of the sort praised by Derek Bok in the opening sentence of this article. Foundations in Russia are still controversial and their future today is even more uncertain than earlier.

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7. Conversations of L. Graham with DFG officials, Bad Godesbug, 4 February 2004. In subsequent discussion with RFBR officials, I. Dezhina was told that originally the major model for the RFBR was the NSF, but that after the RFBR had started its operations, the experience of some other foreign organizations and foundations, including the DFG, were taken into account.
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CHEMISTRY

Lateral Hopping Requires Molecular Rocking

Hiromu Ueba and Martin Wolf

Heterogeneous catalysis is a process by which chemical reactions are fostered on the surfaces of small solid particles. Although many products used in daily life are based on the products of such catalytic processes, our understanding of the underlying elementary steps and reaction dynamics is still far from complete. One of the major goals in surface chemistry, therefore, is to obtain microscopic insight into the dynamics of making and breaking chemical bonds during surface reactions. The challenge is to observe molecular motions and energy redistributions with a time and spatial resolution sufficient to capture the key details. Whereas studies with femtosecond resolution (*1*) and optimum control of chemical reactions (*2*) in the gas phase are well established, a comparable level of sophistication is lacking in the analysis of surface reactions. However, on page 1790 of this issue, Backus *et al.* (*3*) present a direct analysis of the time evolution of the most elementary reaction taking place on a surface: the lateral motion of a molecule (carbon monoxide) on a platinum surface.

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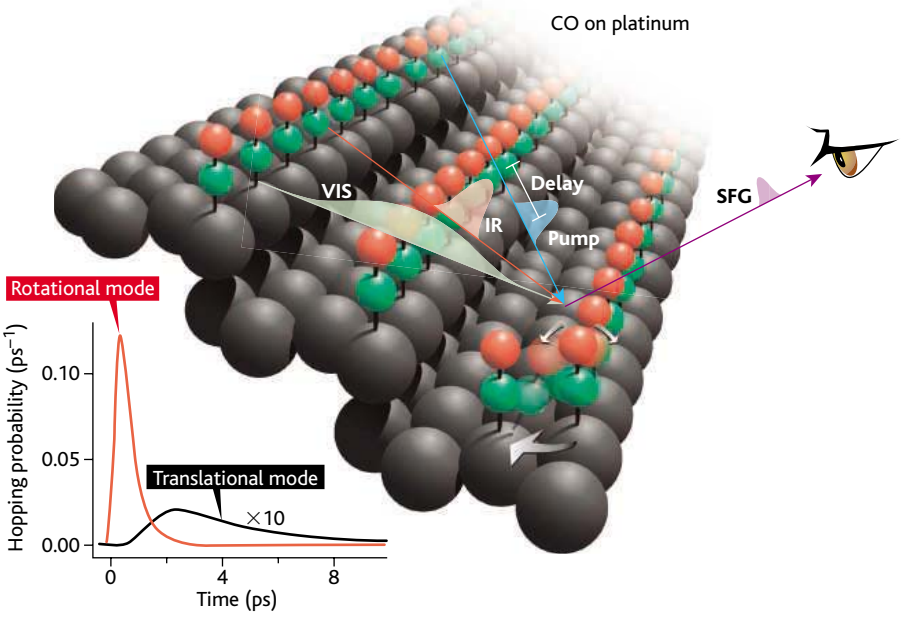
Adsorbate motion on a surface constitutes the most fundamental step for many surface chemical reactions, because it is the primary way for adsorbates to meet a reaction partner or to reach an active site before reaction takes place. As molecules adsorb on specific sites (e.g., on top of surface atoms of the substrate), they move by site-to-site hopping if they can overcome the energetic barrier. Heating the surface is the simplest way to provide the adsorbate with the necessary energy to diffuse. In such a thermally activated process, however, the energy is evenly distributed over all electronic and vibrational degrees of freedom of the adsorbate/surface system. This makes it difficult to identify the elementary reaction steps.

H. Ueba is in the Department of Electronics, Toyama University, 930-8555, Toyama, Japan. M. Wolf is in the Department of Physics, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany. E-mail: ueba@eng.toyama-u.ac.jp

This drawback can be circumvented with the use of femtosecond laser pulses to drive surface diffusion, as demonstrated recently by two independent groups (*4*, *5*). The essence of this approach is that, with ultrafast lasers, heating and cooling occur so rapidly that the different degrees of freedom are not equilibrated, allowing one to distinguish their contribution to the reaction (*6*). In previous reports, the adsorbate motion was not followed in real time, but now Backus *et al.* (*3*) present a real-time study of the lateral motion of molecules on a metal surface. The authors use a pump-probe scheme: A femtosecond pump pulse induces the motion of CO over the surface, and the motion is followed in real time with variably delayed probe pulses (see the figure). The probe consists of femtosecond surface vibrational spectroscopy to look "inside" the CO molecules at the C-O stretch vibration, as these are excited and displaced as a result of femtosecond laser excitation. The clever selection of a nanostructured stepped platinum surface, and the fact that the internal CO stretch frequency depends on the precise location of the CO molecule on this type of surface, enable simultaneous high temporal and spatial resolution.

The adsorbate motion is brought about by heating of the Pt electrons with ultrafast laser pulses. Because the heat capacity of the electrons is very low, they reach temperatures of up to 2500 K for a time of less than 1 ps. These hot electrons, in turn, transfer energy to the adsorbate's low-frequency modes [specifically the Pt-CO stretch, the frustrated translation (FT) mode, and the frustrated rotation (FR) mode]. These modes are characterized by their time-dependent temperature. The typical time scale of energy transfer from the hot electrons in the substrate to the different vibrational modes of CO is characterized by an electron-vibration coupling rate that is different for each mode.

One of the key questions in surface reaction dynamics is: Which mode is associated with which reaction coordinate? For surface diffusion, one would expect that the adsorbate needs sufficient excitation in a direction parallel to the surface for hopping



Carbon monoxide's molecular dance. Schematic view of CO hopping from a step to a terrace site on a platinum surface after excitation with a femtosecond pump pulse (blue). The motion is monitored by ultrafast snapshots of the C-O stretch vibration with sum-frequency generation (SFG) spectroscopy from a delayed pair of infrared (IR) and visible (VIS) laser pulses. Transient changes in the vibrational spectrum give the population of CO molecules on step and terrace sites. (**Inset**) Time evolution of the hopping rate, where lateral motion is induced by the rotational mode (red) or translational mode (black). The experimental observations are compatible only with the ultrafast energy transfer into the rotational mode as a prerequisite for lateral hopping.

to occur from one adsorption site to the next. Intuitively, one might expect that only the translational (FT) mode is responsible. But the authors show that the situation is more complex. In a separate set of experiments, they determine the rate of energy exchange between the Pt electrons and the translational mode, and find that the energy transfer rate occurs much more slowly than the time scale on which actual hopping occurs. Hence, the experimental results are incompatible with the FT mode being the relevant mode responsible for hopping motion. The results are, however, perfectly compatible with the rate at which energy is transferred from the electrons to the FR mode (see the figure, inset).

In this way, Backus *et al*. gain an unprecedented look at the diffusive motion of the CO molecule from one site to the next and obtain detailed information about the rate of energy flow in this model system. In contrast to the widely accepted view that hopping occurs by excitation of multiple quanta in the translational mode to overcome the diffusion barrier, excitation of the rotational mode seems crucial for diffusion. The reaction pathway involves translational motion, which is already thermally populated even if no laser excitation occurs. This precursor state couples to the rotational motion excited by ultrafast coupling to hot substrate electrons and induces diffusion to the next adsorption site on the terrace. The diffusional motion is like a dance in which the CO molecules execute concerted rocking and translational steps (see the figure).

By careful design, Backus *et al*. performed the experiments at 100 K, where thermal excitation of the FT mode is sufficient to reveal the role of the rotational mode, which would otherwise remain obscured by the slower coupling to the FT mode. It should be noted that as both modes have to be excited (thermally or through laser excitation), one expects the translational mode to become rate limiting at lower temperatures, where it is not thermally pre-excited.

The present results demonstrate that for translational motion of diatomic molecules like CO, rotation is required to compensate the tilt of the intramolecular axis relative to the substrate. This involvement of the frustrated rotational mode in surface chemistry appears in a number of surface phenomena: Excitation of the FR mode has been reported to play an important role in the desorption process of CO from Ru(001) (*7*) and from Pt(111) (*8*), even though excitation of the metal-CO stretch mode was expected to be the primary mode for a desorption process. Coupling of vibrational excitation to molecular motion on metal surfaces has been also extensively investigated by observing single-molecule motions and reactions with scanning tunneling microscopy (STM) (*9*–*11*). In these studies, an implicit assumption was that the reaction coordinate simply lies along the coordinate for the frustrated translation. STM-induced hopping may occur by excitation above the barrier as the FT mode is anharmonically coupled to internal stretching modes excited by tunneling electrons from a STM tip. Recently, the diffusion of atomic oxygen on a vicinal Pt(111) surface has been discussed in terms of the indirect excitation of the FT mode via anharmonic coupling to the O-Pt stretch vibration excited by hot electrons (*5*).

Although the inevitable role of mode coupling in surface chemical transformations is unambiguous, for many systems the detailed mechanism of which modes contribute to the motion along the reaction path is an open question. The work by Backus *et al*. shows that a simple one-dimensional view of diffusion is not sufficient and that in general, a concerted motion is occurring. An excellent example of such complex multidimensional dynamics is the rapid diffusion of water dimers on Pd(111), which perform a molecular waltz along the surface (*12*). Backus *et al*. nicely demonstrate that such surface dynamics can be followed by vibrational snapshots directly on the time scale on which the molecular dynamics occur. In the future, such experiments will provide new insights into surface processes on molecular length and time scales, which are essential for a fundamental understanding and control of heterogeneous catalysis and electrochemistry.

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MICROBIOLOGY

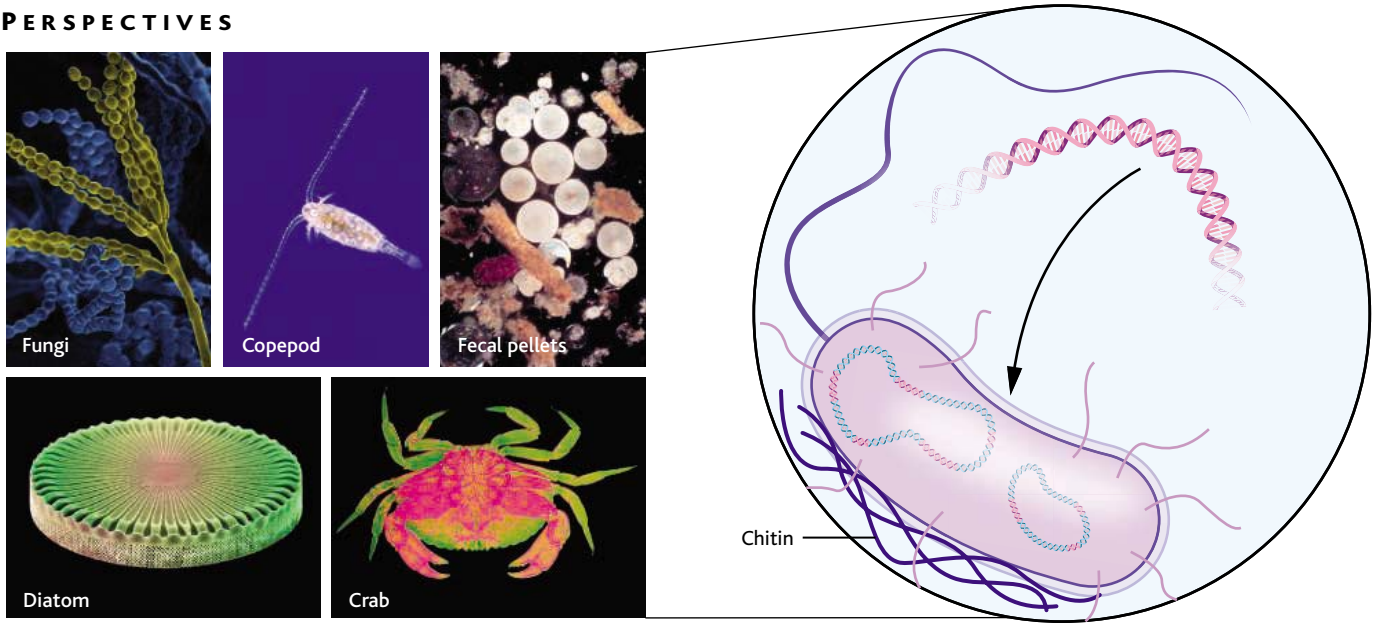
Chitin, Cholera, and Competence

Douglas H. Bartlett and Farooq Azam

The ancient human pathogen *Vibrio cholerae* resides in oceanic, estuarine, and freshwater aquatic environments, where it often attaches to, and feeds on, chitin, the most abundant polymer on Earth after cellulose, and the most abundant polymer in the marine environment (*1*). Chitinous substrates include arthropods and their molts and fecal pellets, some diatoms, and fungi, which are spread over wide geographic and spatial ranges in rivers, estuaries, and oceans (see the figure). The connection between this bacterium and chitin has been a long-standing concern to health scientists for two reasons. First, this association increases the microbe's resistance to acids such as those secreted by the lining of the stomach. Thus, if ingested by drinking contaminated water or improperly cooked shellfish, there is an increased possibility that the microbe will cause cholera, a potentially devastating intestinal infection (*2*). Second, *V. cholerae* biofilms that develop on single, chitin-containing plankton may rise to the level of an infectious dose (*3*). More recently it has been shown that attachment to chitin is favored by *V. cholerae* bacteria that express a surface protein that is also required for infecting humans (*4*).

The Meibom *et al.* study (*5*) on page 1824 in this issue adds a new reason to worry about the *V. cholerae*–chitin connection: the phenomenon of microbial competence, the ability of a bacterium to directly take up DNA present in the environment by the process known as natural DNA transformation. *V. cholerae* is already renowned for its ability to absorb new genes into its genome. But this feature has previously been attributed to bacterial cell-to-cell contacts (conjugation) or virus-mediated events (transduction) (*6*). Even the cholera toxin genes themselves are shared among serotypes and biotypes of *V. cholerae* via bacterial viruses (*7*). The discovery of DNA transformation in *V. cholerae* provides a new mechanism for this organism

The authors are in the Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093–0202, USA. E-mail: dbartlett@ucsd.edu; fazam@ucsd.edu



Bacterial transformation in the aquasphere. Shown are various aquatic chitin-containing organisms and materials. The scales of the images range from ~5 μm (fungal spores) to ~50 μm (diatoms) to ~500 μm (copepod fecal pellets) to ~2 mm (copepods) to many centimeters (crabs). The fecal pellets are the brown objects in the image on the upper right. One route for the entry of such genetically altered *V. cholerae* into the human habitat could be from either drinking water that is contaminated with *V. cholerae* that is attached to chitinaceous structures or from the consumption of organisms that have concentrated *V. cholerae*-chitin.

to effectively acquire genes for adapting to its aquatic habitat or infecting its human host.

Meibom *et al*. developed the hypothesis of competence in *V. cholerae* after observing that chitin induced the production of a protein appendage known as a type IV pilus, a structure sometimes associated with DNA-uptake ability in other bacteria. Chitin oligosaccharides, as well as natural chitin from crab shells, stimulate transformation of bacteria to antibiotic resistance and restore their ability to synthesize amino acids. Components of the type IV pilus are required for competence. The authors examined the role of a chitin-induced gene that regulates natural transformation in other bacteria and compared the genotypes of competence-positive and -negative strains of *V. cholerae*. In an elegant series of genetic experiments, they elucidated three separate regulatory circuits in the bacterium that control transformation. These pathways are stimulated by (i) the availability of chitin; (ii) a lack of nutrients, or by some other stress; or (iii) a sufficiently high bacterial population.

The discovery of chitin-induced competence in *V. cholerae* raises a number of new questions that can be considered from the standpoint of the source of the transforming DNA, and the process and function of DNA uptake. Extracellular DNA concentrations can be in the range of tens of micrograms per liter in the water column between the surface and bottom of the ocean. The concentration is about three orders of magnitude greater in sediments, and higher still within biofilms of various microorganisms (*8*, *9*). Extracellular DNA stabilizes biofilm structure (*9*), and cytidine, a DNA breakdown product, stimulates *V. cholerae* biofilm formation (*10*). In this way, *V. cholerae* cells growing on a chitin surface may benefit from DNA both because of its utility as a biofilm stabilizer and as a source of transformation substrate. This DNA could arise from the cell death and lysis of a subpopulation of bacteria or from membrane vesicles containing DNA that are released from living microbes. DNA release from bacteria can even be stimulated by algae (*11*).

The existence and nature of transformation in *V. cholerae* suggest that this phenomenon could be even more widespread than is currently appreciated. All of the DNA-uptake (or competence) genes identified by Meibom *et al*. have related counterparts within and even beyond other members of this bacterial family, including *Vibrio vulnificus* (the major cause of seafood-related fatalities in the United States) and *Vibrio parahaemolyticus* (another emerging pathogen that causes gastroenteritis). Even more distantly related pathogens such as *Escherichia coli* are worthy of reconsideration. Although *E. coli* is not generally thought to be naturally competent, it has many of the genes associated with the process and can take up plasmid DNA from the environment if concentrations of extracellular calcium are in the millimolar range, as is the case with seawater (*12*).

The requirement for chitin in tranformation imparts a novel twist to the story unfolded by Meibom *et al*. Why chitin? Perhaps it provides a mechanism for scavenging DNA, much as the silica in beach sand does (*13*), thereby creating an opportunity for DNA and bacteria to be brought together onto the two-dimensional surface of a chitin particle. However, because chitin oligosaccharides containing only six *N*-acetylglucosamine residues induce competence, it would seem more likely that the chitin facilitates the presentation of DNA to the cell surface and/or induces the synthesis of the DNA-uptake apparatus.

Another interesting aspect of the work by Meibom *et al.* is that transformation also depends on extracellular signaling among the cells. This is mediated through the production of small organic molecules dubbed "quorum molecules." Quorum signaling in *V. cholerae* controls a growing list of functions, including virulence in both humans and protozoa and biofilm formation (*14*, *15*). It is likely that in the environment, allelopathic interactions among competing bacteria, where a variety of quorum-molecule inhibitors may be produced, will also regulate if, when, and where DNA uptake occurs (*16*, *17*).

Last but not least, why does *V. cholerae* go to the trouble of being transformable in the first place? Transformation can promote genetic diversity, DNA repair, or nutrition. The authors have clearly demonstrated that diversity is at least part of the story. In this regard, transformation could be one reason why some strains of *V. cholerae* have the cholera toxin genes but lack the receptor for a bacteriophage that harbors cholera toxin genes. It could also provide another pathway for *V. cholerae* to acquire new or altered virulence or antibiotic resistance properties. But the value of transformation to the nutrition of bacteria in aquatic environments also deserves consideration. Whatever its source,

DNA can be used by many bacteria as a source of carbon, energy, nitrogen, and phosphorus. The ability to use DNA as a food source confers a dramatic survival advantage to *E. coli* cells in stationary phase over mutant bacteria that lack this ability. As once expressed, "DNA is good eating" (*18*). To what extent *V. cholerae* uses transformation to satisfy this appetite remains to be seen.

Note added in proof: V. cholerae secretes a chitin-binding protein that is also important in colonizing human intestinal epithelial cells (*19*).

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GEOPHYSICS

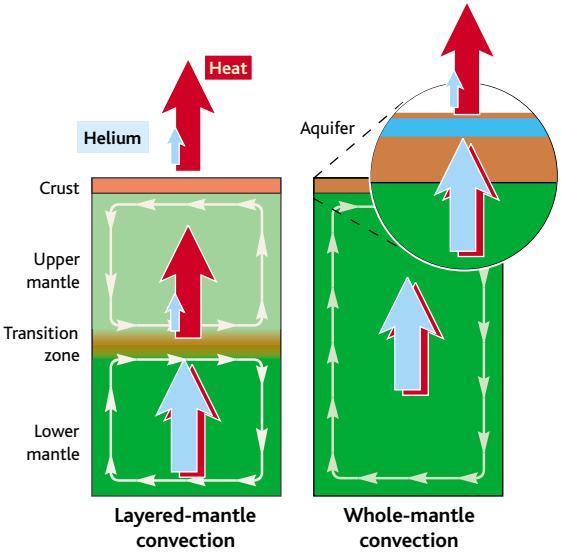
Helium Feels the Heat in Earth's Mantle

Francis Albarède

For nearly two decades, geoscientists held that the terrestrial mantle behaves as two superposed layers separated by a discontinuity at a depth of 660 km. In this picture, convection of mantle material occurs separately in each layer. This view was challenged in the 1990s by evidence from seismic tomography that some subducting plates penetrate almost all the way down to the core-mantle boundary (*1*, *2*). Many of the arguments supporting layered-mantle convection still survive, however, and their impact—notably on chemical geodynamics and on the understanding of terrestrial evolution through geological ages—is still considerable. Most notably, researchers point to the noble gas helium. Reconciling whole-mantle convection with what we know about the abundances of helium isotopes in hotspot and mid-ocean ridge basalts (*3*–*5*) demands a solubility level of this gas in minerals that is rarely considered as acceptable. Second, the terrestrial inventory of the isotope ^{40}Ar created by radioactive decay of ^{40}K leaves too much of this gas unaccounted for. This is commonly interpreted to mean that deep mantle material never came close to the surface to lose its gas content (*6*).

A third very strong argument supporting the model of convection as occurring in separate layers is the apparent imbalance between heat flow and helium loss through the surface of Earth. Most of the energy that sustains convection movements in the mantle and that powers plate tectonics is heat produced by the radioactive nuclides ^{238}U, ^{235}U, and ^{232}Th. These isotopes decay by emitting eight, seven, and six α particles, respectively. These particles are simply the nuclei of ^{4}He (with a small fraction of the energy produced by the decay of ^{40}K). O'Nions and Oxburgh (*7*, *8*) pointed out that the production of heat and helium should be strongly connected but observed that the terrestrial flux of ^{4}He (at the proximity of mid-ocean ridges) only accounted for about 5% of the heat flow. They concluded that somewhere below the surface a conductive boundary layer exists that allows heat to be transferred upward while retaining ^{4}He in the lower reservoir. After examining all the possible boundary layers, notably those located in the crust, they concluded that the helium-heat terrestrial imbalance was best explained by two convecting mantle shells separated by a conductive boundary layer at 660 km. This interpretation was recently reinforced by van Keken *et al.* (*9*), who demonstrated that such an imbalance is not a transient effect of the convective regime. Although the case of the heat/helium imbalance seemed to be clear, further work from an apparently unrelated field would prove it wrong.

A recent article by Castro *et al.* (*10*) is now placing the terrestrial helium-heat imbalance in a very different perspective. These authors constructed a two-dimensional (2D) numerical model of the Carrizo aquifer in Texas and surrounding formations in which the calculated hydraulic pressures, temperatures, and helium concentrations were carefully calibrated against measured field data. They computed local heat and helium fluxes and discovered that for much of this multilayered aquifer system, heat is lost by conduction, whereas helium is transported by advection but only inefficiently because of the low hydraulic conductivities (and thus permeabilities) of the rocks. Under such conditions, the ^{4}He/heat flux ratios are smaller than the "production ratio" in the crust by one to two orders of magnitude. In other words, this is the ^{4}He/heat ratio characteristic of radioactive sources. Recharge of the system with cold meteoric water (that is, from rain or atmospheric condensation), very poor in rare gas contents because it equilibrated with the atmosphere, enhances this effect. Castro *et*



Mantle models. (Left) In layered-mantle convection, heat and helium are separated at the transition zone, which acts as a conductive boundary layer. **(Right)** As shown by Castro *et al.* (*10*), a similar effect is achieved by aquifers in continental crust and by seawater circulating through the oceanic crust. This picture of the crust reconciles the heat–helium flux imbalance with whole-mantle convection.

The author is in the Laboratoire des Sciences de la Terre, Ecole Normale Supérieure de Lyon, 46 Allee d`Italie, 69364 Lyon Cedex 7, France. E-mail: francis.albarede@ens-lyon.fr

al. extend their interpretation to ocean basins in which water injected into the oceanic crust plays the same role as meteoric water in continental aquifers. Their article therefore does justice to the careful warning given by Oxburgh and O'Nions (*8*), who advised that "the systematics of the relation between the fluxes of helium and heat depend on transport processes." This warning was unfortunately lost in the turmoil of the debate between the advocates of whole-mantle versus layered-mantle convection.

One of the cornerstones of layered-mantle convection is therefore weakening. The remaining evidence essentially revolves around our understanding of the properties and inventories of rare gases. We are still awaiting incontrovertible data on rare-gas solubility in mantle minerals and melts, which would consolidate the dominant interpretation of the $^3He/^4He$ evidence in oceanic basalts. Helium-3 is a stable nuclide essentially unaffected by deep-seated radioactive processes. Even if helium turns out to be particularly incompatible, enough undegassed mantle material with high $^3He/^4He$ ratios may be concealed in the lower mantle as streaks interlayered with recycled material (*11*). In addition, the argument based on the inventory of ^{40}Ar in Earth is crucially dependent on our knowledge of the terrestrial concentration of potassium, an element that is known to be extremely volatile during planetary accretion.

By coincidence, the consensus on the heat/helium imbalance at the surface of Earth is being challenged at almost the same moment as new experiments overturn the well-entrenched idea that high $^3He/^4He$ ratios in basalts are the hallmark of primitive mantle. ^3He is a stable isotope of helium, whereas ^4He (α particles) is continuously produced by the decay of uranium and thorium. High $^3He/^4He$ ratios in hotspot basalts (e.g., Hawaii) with respect to mid-ocean ridge basalts have been held as prime evidence that the deep mantle never lost its primordial gases. Measurement of the helium solubility in mantle minerals (*12*) suggests instead that a high $^3He/^4He$ mantle ratio may not be primordial (*3*, *4*) but rather corresponds to residues of earlier stages of melting (*5*, *13, 14*).

We should not let the heritage of layered-mantle convection models fall into oblivion, however. Numerical models of mantle convection repeatedly suggest that radial transport is particularly slow across the 660-km discontinuity and display episodic surges of layered-mantle convection regime (*14*). The article by Castro *et al.* nevertheless provides the first rigorous framework against one of the strongest arguments used to support the role of the 660-km discontinuity as a convection boundary, and the authors should be commended for their work. Further full 2D and 3D models of large well-characterized aquifer systems around the world will soon let us reevaluate the terrestrial heat and helium fluxes and provide a new perspective on the thermal regime of our planet.

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ECOLOGY

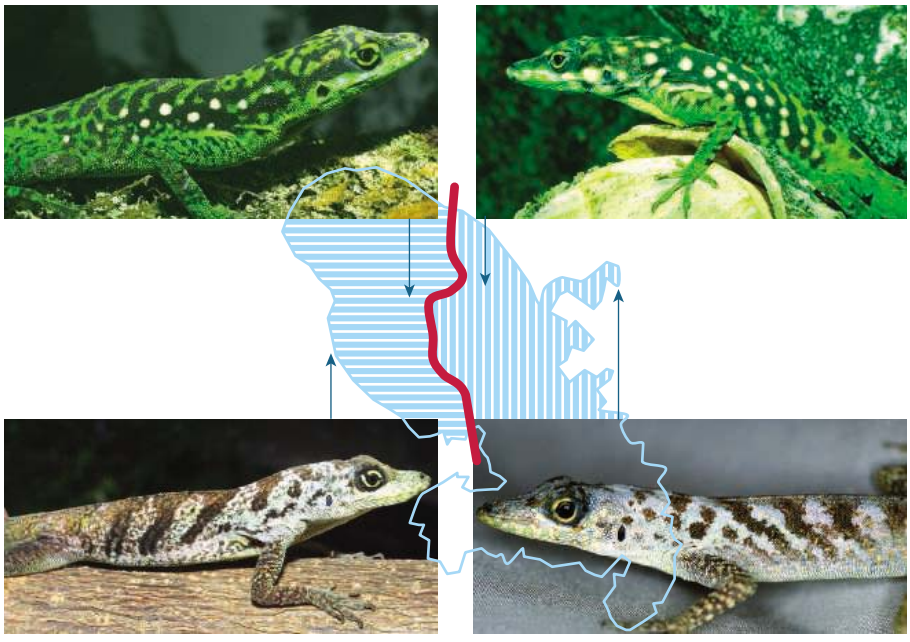
Population Evolution and Island Biogeography

Roger S. Thorpe

In most areas of empirical science, including biology, we take an experimental approach for granted. However, in evolutionary and related studies, large-scale natural or field experiments are rare because the large spatial and temporal scales of evolutionary and biogeographical processes render experimentation problematic. Classically, biogeography is about large-scale pattern: How many species are there on an island? Is the number related to extent of isolation, island size, or complexity of vegetation? For example, why are there so many tree lizard (anole) species on each of the Greater Antilles but only one or two on each of the Lesser Antilles? Is it simply island size that is accountable, even though the small islands are environmentally heterogenous and complex (*1*)? This is not readily subject to direct experimentation. However, these large-scale biogeographic patterns are mediated by small-scale population-level processes. These include ecological processes such as competition between species and habitat usage, and evolutionary processes such as adaptation by natural selection, ancestor-descendant relationships, and speciation (splitting into distinct species, which do not interbreed). Exceptionally, it may be possible to manipulate these fundamental population processes experimentally to gain insight into biogeographic patterns, although even population-level experimentation is difficult. For example, experimental introduction of small Caribbean anoles onto islands, and experimental translocation between large enclosures within islands, have revealed much about the evolutionary and ecological population processes underlying their biogeography. Large-scale translocation of the small tree lizard *Anolis oculatus* (Dominica, Lesser Antilles) has demonstrated the rapid effect of natural selection on a wide range of genetically controlled traits in response to wet or dry habitats (*2*, *3*), thus explaining the nature and cause of the geographic variation. In addition, experimental introduction of *Anolis sagrei* onto Bahamanian islands has shown how predators can alter the behavior, niche usage, and their selection for prey (*4*). These studies also suggested how introduced predators may render their prey more vulnerable to extinction by catastrophes such as hurricanes (*5*).

On page 1807 in this issue, Schoener *et al*. (*6*) show how the survival of resident anoles on islands with introduced predator lizards depends on vegetation height. On islands without the introduced predator, anoles survive better in habitats with shorter vegetation, but on islands with the introduced predator, anoles survive better in habitats with taller vegetation. Island size on its own did not appear to have a significant effect. Hence, the authors take the important step of linking a population process (survival) to a key feature of island biogeography (vegetation type), and this direct demonstration, using a field experi-

The author is in the School of Biological Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, UK. E-mail: r.s.thorpe@bangor.ac.uk



No evidence for the geographic speciation predicted by biogeographic pattern. Broad biogeographic pattern suggests geographic speciation in Lesser Antillean anoles, yet on Martinique, where once separate island species now meet in secondary contact (red line), population-based genetic studies show no evidence of their reproductive isolation (*9*). However, there is strong parallel adaptation to the habitat zonation and some evidence of reduced interbreeding among these habitat types (ecotypes) (*9*). Xeric coastal ecotypes of anoles are brown and striped (lower photographs; arrows indicate locality of origin). Where lineages meet, in the montane rainforest, they show parallel evolution of green montane ecotypes (upper photographs), irrespective of their independent island history and deep molecular phylogenetic divergence.

ment, is novel. This relationship is important because island biogeographic patterns are determined by such population processes. Although population phenomena underlie central aspects of island biogeographic theory, these related disciplines are often pursued without extensive cross-reference. For instance, the species-area relationship (number of species per island compared to size of island) in island biogeography is usually considered in isolation of the population processes that drive speciation. Comparative island biogeographic analysis of the distribution and number of species of native Caribbean island anoles concludes that "within-island" speciation is rare or nonexistent on small islands (*7*). However, this proposition has been made in the absence of population-based genetic evidence, and thus biogeographic pattern is suggesting population process, rather than population process informing biogeographic theory. Moreover, what precisely does "within-island" mean? It has become an axiom of evolutionary ecology that the historical component should be taken into account. This, quite rightly, means that the history of an organism (phylogeny) should be considered. But in island biogeography, should we not also be considering the geological history? We may anthropocentrically regard an island that exists now as a "real" single entity, yet it may have been a single entity for only a brief portion of its history. For example, the island of Martinique in the Lesser Antilles may once have comprised up to five separate islands, some more than 20 million years old, which have spent only a fraction of their existence as part of the single island of Martinique (*8*). Biogeographic patterning suggests that each Lesser Antillean island has one anole species (per clade), implying that each island may have been dominated by allopatric speciation via island colonization. In other words, each island is naturally inhabited by a single anole species by virtue of having evolved into a new species after colonization of a new island because it was geographically, and hence genetically, isolated from its ancestor. This widely accepted view is perhaps immune to challenge because it is virtually impossible to directly test the reproductive isolation of allopatric species. However, it may be possible to test one aspect of this theory by using population-level analysis. The allopatric speciation hypothesis predicts that the endemic forms on ancient islands should act as "good" species and not freely interbreed on contact with a different species. Such secondary contact exists between the anoles from the precursor islands of Martinique (see the figure). However, population-based genetic studies of nuclear gene flow do not support this prediction of no interbreeding on secondary contact of these populations (*9*). Therefore it may be more appropriate for population-based studies to inform biogeographic theory, than for biogeographic pattern to suggest the nature of the population process.

Field experiments on aspects of population biology that are relevant to biogeography are replete with challenges. These problems include maintaining conditions that are as natural as possible such as natural densities of organisms, maintaining discrete experimental units such as small islets or enclosures, having a size and number of replicate units that are large enough to give statistical significance but are still logistically manageable, and having effects that are intense enough to yield results in the time frame of the project and variables with sufficient variance to elicit a response. Island anoles in general, and *A. sagrei* in the islands of the Bahamas in particular, provide a model system that overcomes many of these problems. The small size of anoles, their high population density, numerous studies providing background information on their evolutionary ecology, and their presence on small discrete islands with varying vegetation types make island anoles a valuable model system for both experimental (*1*–*6*) and comparative (*1*, *8*–*10*) studies in evolution and biogeography.

Perhaps the larger challenge will be relating population ecology and population evolution to broader scale spatial variation in the environment. Schoener *et al.* have shown that demographics are certainly sensitive to changes in species composition and that this is habitat specific. Anoles adapt closely to the environmental and climatic zones that exist on even small islands (*1*–*3*, *8*). Can similar experimental studies be used to provide insight into the speed and limits of adaptation to climate change of both individual species and whole communities?

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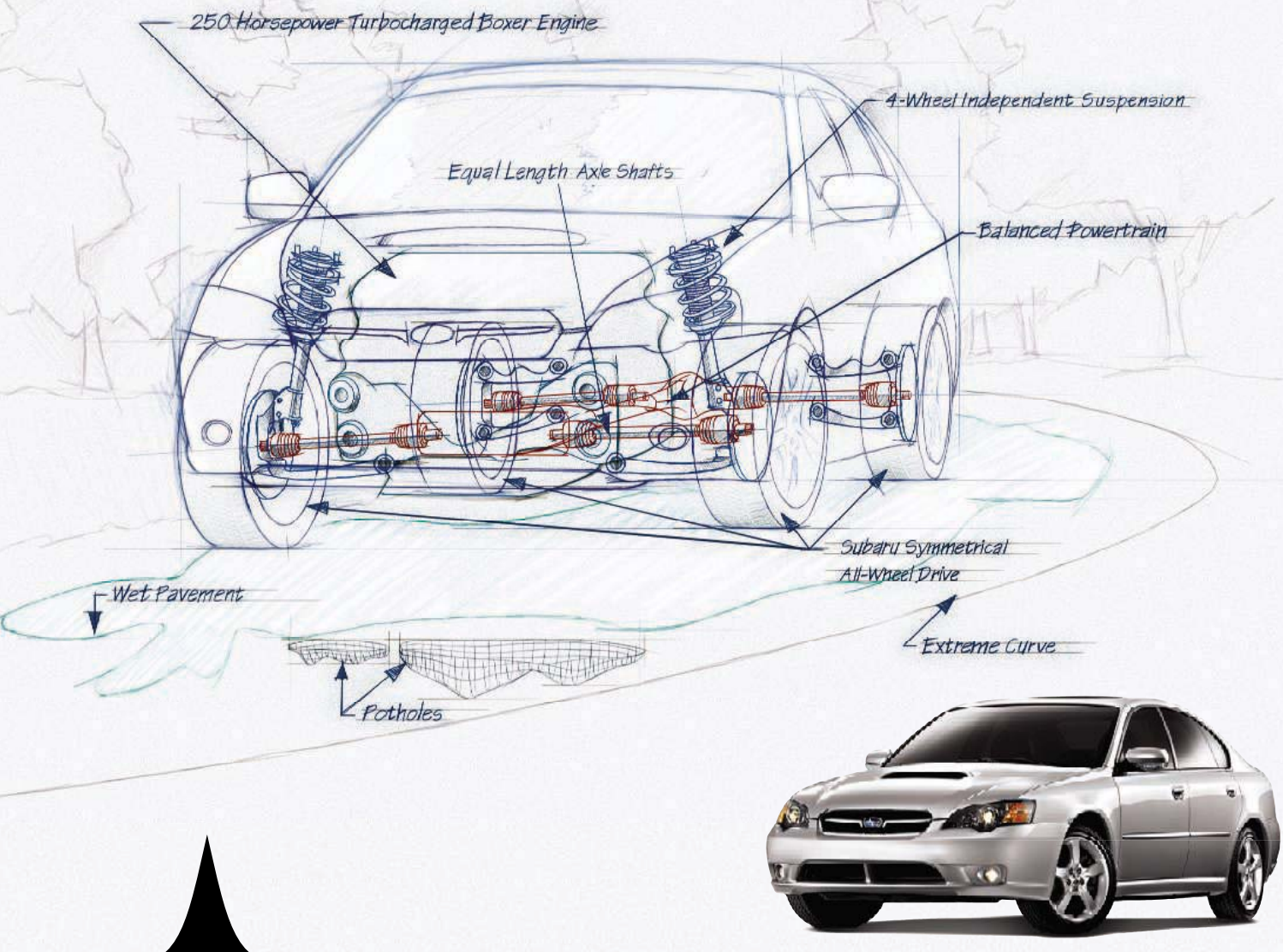

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Aphid Protected from Pathogen by Endosymbiont

Claire L. Scarborough,* Julia Ferrari,*† H. C. J. Godfray

Many invertebrates have intimate associations with bacterial symbionts, and over the past few years, several have been shown to influence their hosts' ability to defend against natural enemies (*1*, *2*). Aphids possess a range of facultative bacterial endosymbionts that may help in defense against parasitoids (*2*) and influence the aphids' ability to use different plant species (*3*). We show here that one of the common facultative symbionts of pea aphid (*Acyrthosiphon pisum*), the bacterium *Regiella insecticola* (synonyms: U-type, PAUS) (*4*), has a major effect on host resistance to a fungal pathogen and lowers the rate of transmission of the fungus. *Regiella* is vertically transmitted during both asexual and sexual reproduction.

A vertically transmitted symbiont (that does not manipulate sex ratio) or one with low levels of horizontal transmission will eventually be lost from a population if carrying it imposes a cost on the host. This loss has led to a search for beneficial effects of the symbiont. By experimentally introducing symbionts, it has been shown that both *Hamiltonella defensa* and *Serratia symbiotica* increased resistance to an aphid parasitoid (*2*), whereas *Regiella* improved the performance of one aphid clone on one of its host plants (*3*).

A major pathogen of aphids is the Entomophthorales fungus *Pandora* (*Erynia*) *neoaphidis*. Infectious spores of the fungus germinate on the aphid cuticle, and mycelia penetrate and fill the aphid's body cavity, leading to death within a few days. Spores are then produced on the cadaver's surface. We previously found a correlation across pea aphid clones between the possession of *Regiella* and resistance to *Pandora* (*6*). To test whether carriage of *Regiella* enhances resistance, we took five facultative symbiont-free pea aphid clones and established novel infections by injecting hemolymph from pea aphids that carried only *Regiella.* After several generations, the aphids were tested for resistance to *Pandora* (*5*).

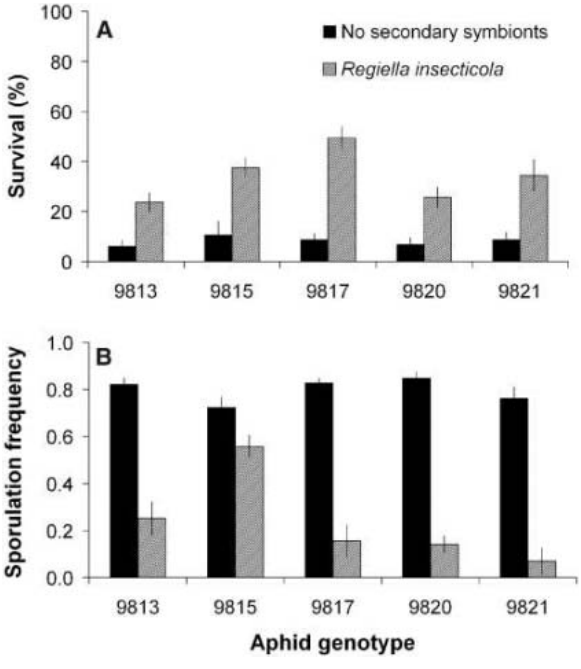

Fig. 1. The facultative endosymbionts *R. insecticola* increase resistance to the entomopathogenic fungus *P. neoaphidis*. Five aphid genotypes were artificially infected with *Regiella*. (**A**) All aphid genotypes had significantly increased survival. (**B**) Even when killed by *P. neoaphidis*, pea aphids harboring *Regiella* produced fewer sporulating cadavers. Means and SEs are shown.

Across all five aphid clones, the association with *Regiella* improved the aphids' ability to survive exposure to the fungal pathogen (Fig. 1A). Symbiont presence (treatment) increased the log odds of survival by 1.8 ± 0.2 (SE), which is highly significant (χ_1^2 = 173.47, P < 0.001). There were significant differences among clones in their susceptibility to fungi (χ_4^2 = 22.39, P = 0.02), although all responded similarly to *Regiella* (clone × treatment, χ_4^2 = 3.46, P = 0.78).

The presence of the symbiont influenced whether the fungus would sporulate successfully on those aphids it had killed (Fig. 1B). *Regiella* reduced the log odds of successful sporulation by –2.7 ± 0.2 (SE) (χ_1^2 = 423.21, P < 0.001). This effect differed significantly among aphid clones (clone, χ_4^2 = 26.98, P < 0.001; clone × treatment, χ_4^2= 56.93, P < 0.001). The interaction between clone and treatment may represent differences among aphid genotypes in their response to *Regiella* or vice versa, because each aphid clone was injected with a different strain of *Regiella*. Failure to sporulate has no bearing on the fate of the individual insect; however, aphids reproduce clonally and are often wingless. Hence, nearby individuals are more likely to be genetically identical, and this will lead to an increase in the aphid's inclusive fitness.

Because *Pandora* is the most common aphid fungal pathogen, our results provide an explanation for *Regiella*'s maintenance in pea aphid populations. But why do not all aphids carry *Regiella*? One possibility is that the advantage of disease resistance varies over time and space and, in *Pandora*'s absence, carrying the symbiont is costly, although no costs to date have been identified. *Regiella* can influence performance on certain host plants (*3*), and, possibly, the net benefits of carrying the symbiont depend both on its effect on resource use and the risk of infection on different food plants. Such an effect might explain why pea aphid clones specialized on *Trifolium* both tend to harbor *Regiella* and show high resistance to *Pandora* (*6*, *7*).

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Natural Environment Research Council Centre for Population Biology, Division of Biology, Imperial College London, Silwood Park Campus, Ascot, Berks, SL5 7PY, UK.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: julia.ferrari@imperial.ac.uk

SLC24A5, a Putative Cation Exchanger, Affects Pigmentation in Zebrafish and Humans

Rebecca L. Lamason,[1]* Manzoor-Ali P.K. Mohideen,[1]† Jason R. Mest,[1] Andrew C. Wong,[1]‡ Heather L. Norton,[6] Michele C. Aros,[1] Michael J. Jurynec,[8] Xianyun Mao,[6] Vanessa R. Humphreville,[1]§ Jasper E. Humbert,[2,9] Soniya Sinha,[2] Jessica L. Moore,[1]‖ Pudur Jagadeeswaran,[10] Wei Zhao,[3] Gang Ning,[7] Izabela Makalowska,[7] Paul M. McKeigue,[11] David O'Donnell,[11] Rick Kittles,[12] Esteban J. Parra,[13] Nancy J. Mangini,[14] David J. Grunwald,[8] Mark D. Shriver,[6] Victor A. Canfield,[4] Keith C. Cheng[1,4,5]¶

Lighter variations of pigmentation in humans are associated with diminished number, size, and density of melanosomes, the pigmented organelles of melanocytes. Here we show that zebrafish *golden* mutants share these melanosomal changes and that *golden* encodes a putative cation exchanger slc24a5 (nckx5) that localizes to an intracellular membrane, likely the melanosome or its precursor. The human ortholog is highly similar in sequence and functional in zebrafish. The evolutionarily conserved ancestral allele of a human coding polymorphism predominates in African and East Asian populations. In contrast, the variant allele is nearly fixed in European populations, is associated with a substantial reduction in regional heterozygosity, and correlates with lighter skin pigmentation in admixed populations, suggesting a key role for the *SLC24A5* gene in human pigmentation.

Pigment color and pattern are important for camouflage and the communication of visual cues. In vertebrates, body coloration is a function of specialized pigment cells derived from the neural crest (*1*). The melanocytes of birds and mammals (homologous to melanophores in other vertebrates) produce the insoluble polymeric pigment melanin. Melanin plays an important role in the protection of DNA from ultraviolet radiation (*2*) and the enhancement of visual acuity by controlling light scatter (*3*). Melanin pigmentation abnormalities have been associated with inflammation and cancer, as well as visual, endocrine, auditory, and platelet defects (*4*).

Despite the cloning of many human albinism genes and the knowledge of over 100 genes that affect coat color in mice, the genetic origin of the striking variations in human skin color is one of the remaining puzzles in biology (*5*). Because the primary ultrastructural differences between melanocytes of dark-skinned Africans and lighter-skinned Europeans include changes in melanosome number, size, and density (*6*, *7*), we reasoned that animal models with similar differences may contribute to our understanding of human skin color. Here we present evidence that the human ortholog of a gene associated with a pigment mutation in zebrafish, *SLC24A5*, plays a role in human skin pigmentation.

The zebrafish *golden* phenotype. The study of pigmentation variants (*5*, *8*) has led to the identification of most of the known genes that affect pigmentation and has contributed to our understanding of basic genetic principles in peas, fruit flies, corn, mice, and other classical model systems. The first recessive mutation studied in zebrafish (*Danio rerio*), *golden* (gol^{b1}), causes hypopigmentation of skin melanophores (Fig. 1) and retinal pigment epithelium (Fig. 2) (*9*). Despite its common use for the calibration of germ-line mutagenesis (*10*), the *golden* gene remained unidentified.

The *golden* phenotype is characterized by delayed and reduced development of melanin pigmentation. At approximately 48 hours postfertilization (hpf), melanin pigmentation is evident in the melanophores and retinal pigment epithelium (RPE) of wild-type embryos (Fig. 2A) but is not apparent in *golden* embryos (Fig. 2B). By 72 hpf, *golden* melanophores and RPE begin to develop pigmentation (Fig. 2, F and G) that is lighter than that of wild type (Fig. 2, D and E). In adult zebrafish, the melanophore-rich dark stripes are considerably lighter in *golden* compared with wild-type animals (Fig. 1, A and B). In regions of the ventral stripes where melanophore density is low enough to distinguish individual cells, it is apparent that the melanophores of *golden* adults are less melanin-rich than those in wild-type fish (Fig. 1, A and B).

Transmission electron microscopy was used to determine the cellular basis of *golden* hypopigmentation in skin melanophores and RPE of ~55-hpf wild-type and *golden* zebrafish. Wild-type melanophores contained numerous, uniformly dense, round-to-oval melanosomes (Fig. 1, C and E). The melanophores of *golden* fish were thinner and contained fewer melanosomes (Fig. 1D). In addition, *golden* melanosomes were smaller, less electron-dense, and irregularly shaped (Fig. 1F). Comparable differences between wild-type and *golden* melanosomes were present in the RPE (fig. S1, A and B).

Dysmorphic melanosomes have also been reported in mouse models of Hermansky-Pudlak syndrome (HPS) (*11*, *12*). Because HPS is characterized by defects in platelet-dense granules and lysosomes as well as melano-

[1]Jake Gittlen Cancer Research Foundation, Department of Pathology; [2]Intercollege Graduate Degree Program in Genetics; [3]Department of Health Evaluation Sciences; [4]Department of Pharmacology; [5]Department of Biochemistry and Molecular Biology, The Pennsylvania State University College of Medicine, Hershey, PA 17033, USA. [6]Department of Anthropology, [7]The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA. [8]Department of Human Genetics, University of Utah, Salt Lake City, UT 84112, USA. [9]Department of Genetics, Weis Center for Research, Danville, PA 17822, USA. [10]Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA. [11]Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland. [12]Department of Molecular Virology, Immunology and Medical Genetics, Ohio State University, Columbus, OH 43210, USA. [13]Department of Anthropology, University of Toronto at Mississauga, Mississauga, ON L5L 1C6, Canada. [14]Department of Anatomy and Cell Biology, Indiana University School of Medicine-Northwest, Gary, IN 46408, USA.

*Present address: The Graduate Program in Immunology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.
†Present address: Health System Management Center, Case Western Reserve University, Cleveland, OH 44106, USA.
‡Present address: Department of Human Genetics, Emory University, Atlanta, GA 30322, USA.
§Present address: The Pennsylvania State University College of Medicine, H060, 500 University Drive, Hershey, PA 17033, USA.
‖Present address: Department of Biology, University of South Florida, Tampa, FL 33620, USA.
¶To whom correspondence should be addressed. E-mail: kcheng@psu.edu

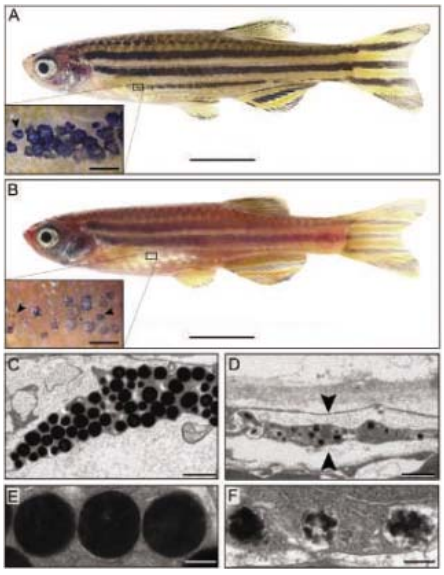


Fig. 1. Phenotype of *golden* zebrafish. Lateral views of adult wild-type (**A**) and *golden* (**B**) zebrafish. Insets show melanophores (arrowheads). Scale bars, 5 mm (inset, 0.5 mm). gol^{b1} mutants have melanophores that are, on average, smaller, more pale, and transparent. Transmission electron micrographs of skin melanophore from 55-hpf wild-type (**C** and **E**) and gol^{b1} (**D** and **F**) larvae. gol^{b1} skin melanophores (arrowheads show edges) are thinner and contain fewer melanosomes than do those of wild type. Melanosomes of gol^{b1} larvae are fewer in number, smaller, less-pigmented, and irregular compared with wild type. Scale bars in (C) and (D), 1000 nm; in (E) and (F), 200 nm.

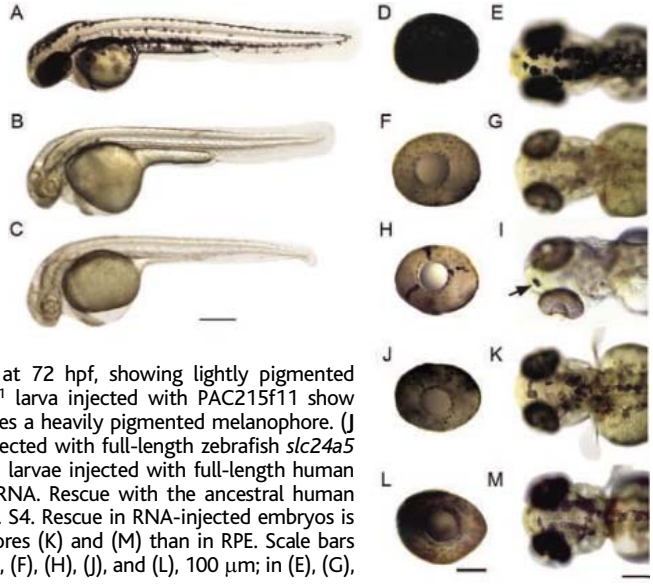


Fig. 2. Rescue and morpholino knockdown establish *slc24a5* as the *golden* gene. Lateral views of 48-hpf (**A**) wild-type and (**B**) gol^{b1} zebrafish larvae. (**C**) 48-hpf wild-type larva injected with morpholino targeted to the translational start site of *slc24a5* phenocopies the gol^{b1} mutation. Lateral view of eye (**D**) and dorsal view of head (**E**) of 72-hpf wild-type embryos. (**F** and **G**) gol^{b1} pigmentation pattern at 72 hpf, showing lightly pigmented cells. (**H** and **I**) 72 hpf gol^{b1} larva injected with PAC215f11 show mosaic rescue; arrow identifies a heavily pigmented melanophore. (**J** and **K**) 72-hpf gol^{b1} larva injected with full-length zebrafish *slc24a5* RNA. (**L** and **M**) 72-hpf gol^{b1} larvae injected with full-length human European (Thr^{111}) *SLC24A5* RNA. Rescue with the ancestral human allele (Ala^{111}) is shown in fig. S4. Rescue in RNA-injected embryos is more apparent in melanophores (K) and (M) than in RPE. Scale bars in [(A) to (C)], 300 μm; in (D), (F), (H), (J), and (L), 100 μm; in (E), (G), (I), (K), and (M), 200 μm.

somes, we examined whether the *golden* mutation also affects thrombocyte function in the zebrafish. A comparison of *golden* and wild-type larvae in a laser-induced arterial thrombosis assay (*13*) revealed no significant difference in clotting time (35 versus 30 s). The *golden* phenotype thus appears to be restricted to melanin pigment cells in zebrafish.

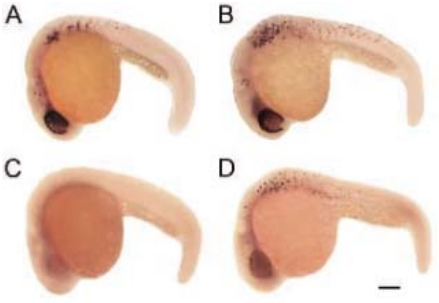
The zebrafish *golden* gene is *slc24a5/nckx5*. Similarities between zebrafish *golden* and light-skinned human melanosomes suggested that the positional cloning of *golden* might lead to the identification of a phylogenetically conserved class of genes that regulate melanosome morphogenesis. Positional cloning, morpholino knockdown, DNA and RNA rescue, and expression analysis were used to identify the gene underlying the *golden* phenotype. Linkage analysis of 1126 homozygous gol^{b1} embryos (representing 2252 meioses) revealed a single crossover between *golden* and microsatellite marker z13836 on chromosome 18. This map distance of 0.044 centimorgans (cM) [95% confidence interval (CI), 0.01 to 0.16 cM] corresponds to a physical distance of about 33 kilobases (kb) (using 1 cM = 740 kb) (*14*). Marker z9484 was also tightly linked to *golden* but informative in fewer individuals; no recombinants between z9484 and *golden* were identified in 468 embryos (95% CI, distance <0.32 cM). Polymerase chain reaction (PCR) analysis of a γ-radiation–induced deletion allele, gol^{b13} (*15*), showed a loss of markers z10264, z9404, z928, and z13836, but not z9484 (fig. S2A). Screening of a zebrafish genomic library (*16*) led to the identification of a clone (PAC215f11) containing both z13836 and z9484 within an ~85-kb insert. Microinjection of PAC215f11 into *golden* embryos produced mosaic rescue of wild-type pigmentation in embryonic melanophores and RPE (Fig. 2, H and I), indicating the presence of a functional *golden* gene within this clone.

Shotgun sequencing, contig assembly, and gene prediction revealed two partial and three complete genes within PAC215f11 (fig. S2B): the 3′ end of a thrombospondin-repeat–containing gene (*flj13710*), a putative potassium-dependent sodium/calcium exchanger (*slc24a5*), myelin expression factor 2 (*myef2*), a cortexin homolog (*ctxn2*), and the 5′ end of a sodium/potassium/chloride cotransporter gene (*slc12a1*). We screened each candidate gene using morpholino antisense oligonucleotides directed against either the initiation codon (*17*) or splice donor junctions (*18*). Only embryos injected with a morpholino targeted to *slc24a5* (either of two splice-junction morpholinos or one start codon morpholino) successfully phenocopied *golden* (Fig. 2C). In rescue experiments, injection of full-length, wild-type *slc24a5* transcript into homozygous gol^{b1} embryos led to the partial restoration of wild-type pigmentation in both melanophores and RPE (Fig. 2, J and K). Taken together, these results confirm the identity of *golden* as *slc24a5*.

To identify the mutation in the gol^{b1} allele, we compared complementary DNA (cDNA) and genomic sequence from wild-type and gol^{b1} embryos. A C→A nucleotide transversion that converts $Tyr^{\cdot 208}$ to a stop codon was found in gol^{b1} cDNA clones (GenBank accession number AY682554) and verified by sequencing gol^{b1} genomic DNA (fig. S3C). Conceptual translation of the mutant sequence predicts the truncation of the *golden* polypeptide to about 40% of its normal size, with loss of the central hydrophilic loop and the C-terminal cluster of potential transmembrane domains.

In wild-type embryos, the RNA expression pattern of *slc24a5* (Fig. 3A) resembled that of the melanin biosynthesis marker *dct* (Fig. 3B), consistent with expression of *slc24a5* in melanophores and RPE. In contrast, *slc24a5* expression was nearly undetectable in *golden* embryos (Fig. 3C), the expected result of nonsense-mediated mRNA decay (*19*). The extent of protein deletion associated with the gol^{b1} mutation, together with its low expression, suggests that gol^{b1} is a null mutation. The persistence of melanosome morphogenesis, despite likely absence of function, suggests that *golden* plays a modulatory rather than essential role in the formation of the melanosome. The pattern of *dct* expression seen in *golden* embryos (Fig. 3D) resembles that of wild-type embryos, indicating that the *golden* mutation does not affect the generation or migration of melanophores.

Conservation of *golden* gene structure and function in vertebrate evolution. Comparison of *golden* cDNA (accession number AY538713) to genomic (accession number AY581204) sequences shows that the wild-type gene contains nine exons (fig. S2C) encoding 513 amino acids (fig. S3A).



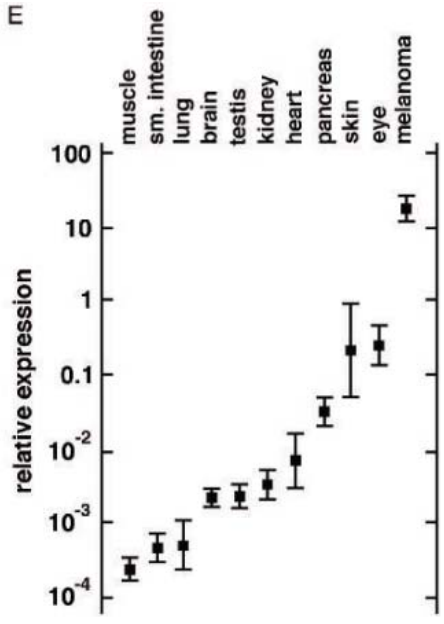


Fig. 3. Expression of *slc24a5* in zebrafish embryos and adult mouse tissues. The expression of *slc24a5* (**A**) and *dct* (**B**) in melanophores and RPE of a 24-hpf wild-type zebrafish larva show similar patterns. (**C**) gol^{b1} larvae lack detectable *slc24a5* expression. (**D**) *dct* expression in 24-hpf gol^{b1} larva is similar to that in wild type. Scale bar, 200 μm. (**E**) Quantitative RT-PCR analysis of *Slc24a5* expression in mouse tissues and B16 melanoma. Expression was normalized using the ratio between *Slc24a5* and the control transcript, RNA polymerase II (*Polr2e*).

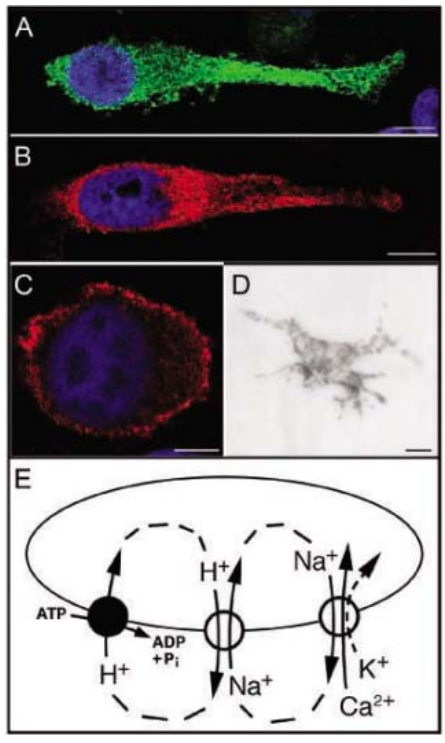


Fig. 4. Subcellular localization of slc24a5. Human MNT1 cells transfected with (**A**) GFP-tagged zebrafish slc24a5 (green) and (**B**) HA-tagged slc24a5 (red) clearly show intracellular expression. (**C**) HA-tagged D3 dopamine receptor localizes to the plasma membrane in MNT1 cells (red). 4′,6′-diamidino-2-phenylindole (DAPI) counterstain was used to visualize nuclei (blue). Scale bars in (A) and (B), 10 μm; in (C), 5 μm. (**D**) Rescue of dark pigmentation in a melanophore of a *golden* embryo by HA-tagged slc24a5. These dark cells appear in *golden* embryos injected with the HA-tagged construct, but not in mock-injected embryos. Scale bar, 10 μm. (**E**) Model for calcium accumulation in melanosomes. Protons are actively transported into the melanosome by the V-ATPase (left). The proton electrochemical potential gradient drives sodium uptake via the sodium (Na^+)/proton (H^+) exchanger (center). Sodium efflux is coupled to calcium uptake by the slc24a5 polypeptide (right). If potassium (dashed arrow) is cotransported with calcium, it must either accumulate within the melanosome or exit by means of additional transporters (not depicted). P_i, inorganic phosphate; ADP, adenosine diphosphate.

BLAST searches revealed that the protein is most similar to potassium-dependent sodium/calcium exchangers (encoded by the *NCKX* gene family), with highest similarity (68 to 69% amino acid identity) to murine *Slc24a5* (accession number BAC40800) and human *SLC24A5* (accession number NP_995322) (fig. S3B). The zebrafish *golden* gene shares less similarity with other human *NCKX* genes (35 to 41% identity to *SLC24A*1 to *SLC24A4*) or sodium/calcium exchanger (*NCX*) genes (26 to 29% identity to *SLC8A1* to *SLC8A3*). Shared intron/exon structure and gene order (*slc24a5*, *myef2*, *ctxn2*, and *slc12a5*) between fish and mammals further supports the conclusion that the zebrafish *golden* gene and *SLC24A5* are orthologs. The high sequence similarity among the orthologous sequences from fish and mammals (fig. S3A) suggested that function may also be conserved. The ability of human *SLC24A5* mRNA to rescue melanin pigmentation when injected into *golden* zebrafish embryos (Fig. 2, L and M, and fig. S4) demonstrated functional conservation of the mammalian and fish polypeptides across vertebrate evolution.

Tissue-specific expression of *Slc24a5*. Quantitative reverse transcriptase PCR (RT-PCR) was used to examine *Slc24a5* expression in normal mouse tissues and in the B16 melanoma cell line (Fig. 3E). *Slc24a5* expression varied 1000-fold between tissues, with concentrations in skin and eye at least 10-fold higher than in other tissues. The mouse melanoma showed ~100-fold greater expression of *Slc24a5* compared with normal skin and eye. These results suggest that mammalian *Slc24a5*, like zebrafish *golden*, appears to be highly expressed in melanin-producing cells.

Model for the role of SLC24A5 in pigmentation. SLC24A5 shares with other members of the protein family a potential hydrophobic signal sequence near the amino terminus and 11 hydrophobic segments, forming two groups of potential transmembrane segments separated by a central cytoplasmic domain. This structure is consistent with membrane localization, although the specific topology of these proteins remains controversial (*20*). Elucidation of the specific role of this exchanger in melanosome morphogenesis requires knowledge of its subcellular localization and transport properties. Although previously characterized members of the NCKX and NCX families have been shown to be plasma membrane proteins (*21*), the melanosomal phenotype of *golden* suggested the possibility that the slc24a5 protein resides in the melanosome membrane. To distinguish between these alternatives, confocal microscopy was used to localize green fluorescent protein (GFP)– and hemagglutinin (HA)-tagged derivatives of zebrafish slc24a5 in MNT1, a constitutively pigmented human melanoma cell line (*22*). Both slc24a5 fusion proteins displayed an intracellular pattern of localization (Fig. 4, A and B), which is distinct from that of a known plasma membrane control (Fig. 4C). The HA-tagged protein showed phenotypic rescue of the *golden* phenotype (Fig. 4D), indicating that tag addition did not abrogate its function. Taken together, these results indicate that the slc24a5 protein functions in intracellular, membrane-bound structures, consistent with melanosomes and/or their precursors.

Several observations suggest a model for the involvement of slc24a5 in organellar calcium uptake (Fig. 4E). First, the intracellular localization of the slc24a5 protein suggests that it affects organellar, rather than cytoplasmic, calcium concentrations, in contrast with other members of the NCX and NCKX families. Second, the accumulation of calcium in mammalian melanosomes appears to occur in a transmembrane pH gradient–dependent manner (*23*). Third, several subunits of the vacuolar proton adenosine triphosphatase (V-ATPase) and at least two intracellular sodium/proton exchangers have also been localized to melanosomes (*24*, *25*). In the model, active transport of pro-

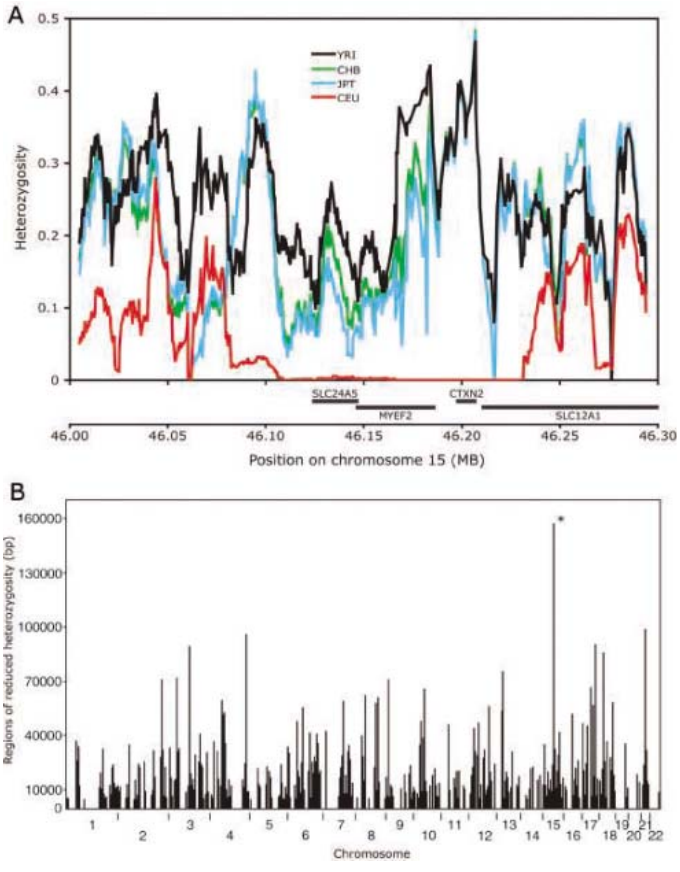


Fig. 5. Region of decreased heterozygosity in Europeans on chromosome 15 near *SLC24A5*. (**A**) Heterozygosity for four HapMap populations plotted as averages over 10-kb intervals. YRI, Yoruba from Ibadan, Nigeria (black); CHB, Han Chinese from Beijing (green); JPT, Japanese from Tokyo (light blue); CEU, CEPH (Foundation Jean Dausset–Centre d'Etude du Polymorphisme Humain) population of northern and western European ancestry from Utah (red). The data are from HapMap release 18 (phase II). (**B**) Distribution in genome of extended regions with low heterozygosity in the CEU sample. Only regions larger than 5 kb in which all SNPs have minor allele frequencies ≤0.05 and which contained at least one SNP with a population frequency difference between CEU and YRI of greater than 0.75 were plotted. Regions were divided at gaps between genotyped SNPs exceeding 10 kb. The data are from HapMap release 16c.1. An asterisk marks the region containing SLC24A5 within 15q21.

tons by the V-ATPase is coupled to slc24a5-mediated calcium transport via a sodium/proton exchanger. The melanosomal phenotype of the zebrafish *golden* mutant suggests that the calcium accumulation predicted by the model plays a role in melanosome morphogenesis and melanogenesis. The observations that processing of the melanosomal scaffolding protein pmel17 is mediated by a furin-like protease (*26*) and that furin activity is calcium-dependent (*27*) are consistent with this view. The role of pH in melanogenesis has been studied far more extensively than that of calcium, with alterations in pH affecting both the maturation of tyrosinase and its catalytic activity (*25*, *28*). The interdependence of proton and calcium gradients in the model may thus provide a second mechanism, in addition to calcium-dependent melanosome morphogenesis, by which the activity of slc24a5 might affect melanin pigmentation.

Role of *SLC24A5* in human pigmentation. To evaluate the potential impact of *SLC24A5* on the evolution of human skin pigmentation, we looked for polymorphisms within the gene. We noted that the G and A alleles of the single nucleotide polymorphism (SNP) rs1426654 encoded alanine or threonine, respectively, at amino acid 111 in the third exon of *SLC24A5*. This was the only coding SNP within SLC24A5 in the International Haplotype Map (HapMap) release 16c.1 (*29*). Sequence comparisons indicate the presence of alanine at the corresponding position in all other known members of the *SLC24* (*NCKX*) gene family (fig. S5). The SNP rs1426654 had been previously shown to rank second (after the FY null allele at the Duffy antigen locus) in a tabulation of 3011 ancestry-informative markers (*30*). The allele frequency for the Thr^{111} variant ranged from 98.7 to 100% among several European-American population samples, whereas the ancestral alanine allele (Ala^{111}) had a frequency of 93 to 100% in African, Indigenous American, and East Asian population samples (fig. S6) (*29*, *30*). The difference in allele frequencies between the European and African populations at rs1426654 ranks within the top 0.01% of SNP markers in the HapMap database (*29*), consistent with the possibility that this SNP has been a target of natural or sexual selection.

A striking reduction in heterozygosity near *SLC24A5* in the European HapMap sample (Fig. 5A) constitutes additional evidence for selection. The 150-kb region on chromosome 15 that includes *SLC24A5, MYEF2, CTNX2*, and part of *SLC12A1* has an average heterozygosity of only 0.0072 in the European sample, which is considerably lower than that of the non-European HapMap samples (0.175 to 0.226). This region, which contains several additional SNPs with high-frequency differences between populations, was the largest contiguous autosomal region of low heterozygosity in the European (CEU) population sample (Fig. 5B). This pattern of variation is consistent with the occurrence of a selective sweep in this genomic region in a population ancestral to Europeans. For comparison, diminished heterozygosity is seen in a 22-kb region encompassing the 3′ half of *MATP* (*SLC45A2*) in European samples, and more detailed analysis of this genomic region shows evidence for a selective sweep (*31*). However, the gene for agouti signaling protein (ASIP), which is known to be involved in pigmentation differences (*32*), shows no such evidence.

The availability of samples from two recently admixed populations, an African-American and an African-Caribbean population, allowed us to determine whether the rs1426654 polymorphism in *SLC24A5* correlates with skin pigmentation levels, as measured by reflectometry (*33*). Regression analysis using ancestry and *SLC24A5* genotype as independent variables revealed an impact of *SLC24A5* on skin pigmentation (Fig. 6). Despite considerable overlap in skin pigmentation between genotypic groups, regression lines for individuals with GG versus AG and GG versus AA genotypes were separated by about 7 and 9.5 melanin units, respectively (Fig. 6A). These differences are more evident in plots of skin pigmentation separated by genotype (Fig. 6B). *SLC24A5* genotype contributed an estimated 7.5, 9.5, or 11.2 melanin units to the differences in melanin pigmentation among African-Americans and African-Caribbeans in the dominant, unconstrained (additive effect plus dominance deviation), or additive models, respectively.

The computer program ADMIXMAP provides a test of gene effect that corrects for potential biases caused by uncertainty in the estimation of admixture from marker data (*34*). Score tests for association of melanin index with the *SLC24A5* polymorphism were significant in both African-American ($P = 3 \times 10^{-6}$) and African-Caribbean population subsamples ($P = 2 \times 10^{-4}$). The effect of *SLC24A5* on melanin index is between 7.6 and 11.4 melanin units (95% confidence limits). The data suggest that the skin-lightening effect of the A (Thr) allele is partially dominant to the G (Ala) allele. Based on the average pigmentation difference between European-Americans and African-Americans of about 30 melanin units (*33*),

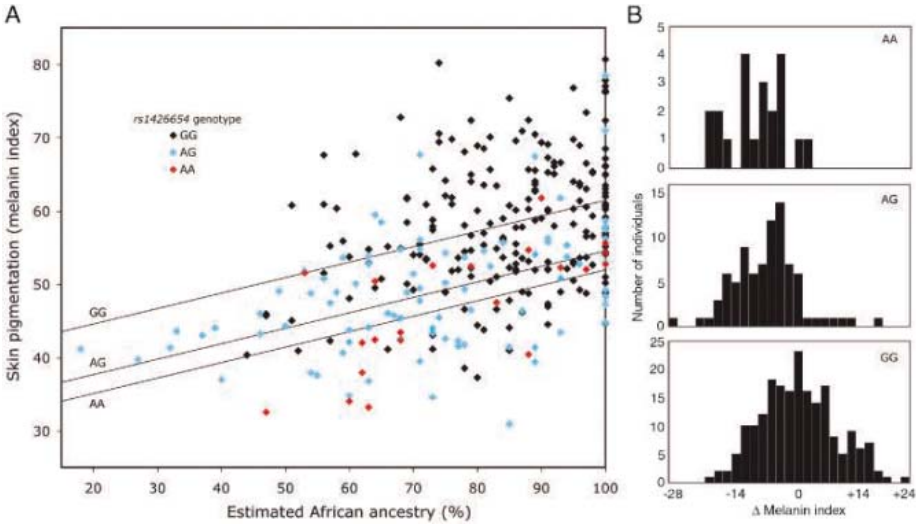


Fig. 6. Effect of *SLC24A5* genotype on pigmentation in admixed populations. (**A**) Variation of measured pigmentation with estimated ancestry and *SLC24A5* genotype. Each point represents a single individual; *SLC24A5* genotypes are indicated by color. Lines show regressions, constrained to have equal slopes, for each of the three genotypes. (**B**) Histograms showing the distribution of pigmentation after adjustment for ancestry for each genotype. Values shown are the difference between the measured melanin index and the calculated GG regression line ($y = 0.2113x + 30.91$). The corresponding uncorrected histograms are shown in fig. S7. Mean and SD (in parentheses) are given as follows: for GG, 0 (8.5), $n = 202$ individuals; for AG, –7.0 (7.4), $n = 85$; for AA, –9.6 (6.4), $n = 21$.

our results suggest that *SLC24A5* explains between 25 and 38% of the European-African difference in skin melanin index.

Relative contributions of *SLC24A5* and other genes to human pigment variation. Our estimates of the effect of *SLC24A5* on pigmentation are consistent with previous work indicating that multiple genes must be invoked to explain the skin pigmentation differences between Europeans and Africans (*5*, *35*). Significant effects of several previously known pigmentation genes have been demonstrated, including those of *MATP* (*36*), *ASIP* (*32*), *TYR* (*33*), and *OCA2* (*33*), but the magnitude of the contribution has been determined only for *ASIP*, which accounts for ≤4 melanin units (*32*). *MATP* may have a larger effect (*37*), but it can be concluded that much of the remaining difference in skin pigmentation remains to be explained.

Variation of skin, eye, and hair color in Europeans, in whom a haplotype containing the derived Thr^{111} allele predominates, indicates that other genes contribute to pigmentation within this population. For example, variants in *MC1R* have been linked to red hair and very light skin [reviewed in (*37*)], whereas *OCA2* or a gene closely linked to it is involved in eye color (*7*, *38*). The lightening caused by the derived allele of *SLC24A5* may be permissive for the effect of other genes on eye or hair color in Europeans.

Because Africans and East Asians share the ancestral Ala^{111} allele of rs1426654, this polymorphism cannot be responsible for the marked difference in skin pigmentation between these groups. Although we cannot rule out a contribution from other polymorphisms within this gene, the high heterozygosity in this region argues against a selective sweep in a population ancestral to East Asians. It will be interesting to determine whether the polymorphisms responsible for determining the lighter skin color of East Asians are unique to these populations or shared with Europeans.

The importance of model systems in human gene discovery. Our identification of the role of *SLC24A5* in human pigmentation began with the positional cloning of a mutation in zebrafish. Typically, the search for genes associated with specific phenotypes in humans results in multiple potential candidates. Our results suggest that distinguishing the functional genes from multiple candidates may require a combination of phylogenetic analysis, nonmammalian functional genomics, and human genetics. Such cross-disciplinary approaches thus appear to be an effective way to mine societal benefit from our investment in the human genome.

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Complete Photo-Induced Breakup of the H_2 Molecule as a Probe of Molecular Electron Correlation

Wim Vanroose,[1] Fernando Martín,[2] Thomas N. Rescigno,[3] C. William McCurdy[3,4]

Despite decades of progress in quantum mechanics, electron correlation effects are still only partially understood. Experiments in which both electrons are ejected from an oriented hydrogen molecule by absorption of a single photon have recently demonstrated a puzzling phenomenon: The ejection pattern of the electrons depends sensitively on the bond distance between the two nuclei as they vibrate in their ground state. Here, we report a complete numerical solution of the Schrödinger equation for the double photoionization of H_2. The results suggest that the distribution of photoelectrons emitted from aligned molecules reflects electron correlation effects that are purely molecular in origin.

Chemical physics has long sought deeper insight into correlation effects because they lie at the heart of understanding atomic and molecular structure. Because quantum mechanics does not offer an analytic solution of the wave function for any physical system with three or more charged particles, electron correlation is a challenge to model mathematically even in the simplest atomic and molecular systems. Processes that put two electrons into the continuum, particularly near the ionization threshold, can offer unique insight into the nature of electron correlation. In particular, complete breakup of a hydrogen molecule after the absorption of only one photon, $h\nu + H_2 \rightarrow p + p + e^- + e^-$, is a pure manifestation of the correlated motion of all the particles in the system, because the energy of a single photon must be shared by all the outgoing particles. Recent imaging techniques have allowed the simultaneous observation of the charged particles that emerge in the breakup of atoms and molecules after a collision with photons or other particles (*1*–*4*). Such detailed data (*5*) offer substantial insight into the nature of electronic correlation. However, a precise quantum theoretical treatment is needed to unambiguously unravel the possible origins of the effects observed (*6*). We report such a complete computational treatment of molecular double ionization.

In double photoionization, two electrons are emitted from an atom or a molecule after absorption of a single photon. The observed pattern of electron ejection is a reflection of the competition between the effects of electron-nuclear attraction, electron-electron repulsion, and the acceleration of the electrons by the electric field in the direction of its polarization. To the degree that the electron ejection pattern is different when the two electrons are in a chemical bond (in the H_2 molecule versus in the helium atom), these experiments can probe the nature of correlation in that bond. Are the observed angular patterns simply a manifestation of the intrinsic molecular symmetries of the initial and final states, or do they reveal more specific information about the dynamics of the ejection process? The variation of the two-electron ejection pattern with respect to initial orientation and internuclear distance of the molecule—either in precise calculations or in experiments—allows us to investigate these questions.

In the experiments recently reported by Weber *et al.* (*5*), linearly polarized 75.5-eV synchrotron radiation was used to study single-photon–induced fragmentation of molecular deuterium (*7*). By measuring the final momenta of both nuclei and the ejected electrons in coincidence, they were able to relate the angular pattern of the ejected electrons to a particular internuclear separation in the molecular ground state at the instant of photon absorption. Weber *et al.* demonstrated experimentally that this essentially classical interpretation of nuclear motion [consistent with the "reflection principle" for photodissociation spectra (*8*)] is valid for the conditions of their experiment. For certain geometries they found marked differences in the angular patterns of ejection of the electrons with small changes in the nuclear momenta, and they speculated that this effect was the signature of changes in electron correlation with bond distance. It is not surprising, of course, that electron correlation in a molecule should change with bond distance. However, Weber *et al.* asserted that small changes in bond distance result in major changes in the angular distributions of the ejected electrons.

There is, however, a complication. The two electrons are ejected suddenly from the vibrating molecule with the nuclei a particular distance apart. A coulomb explosion follows, and the nuclei separate with a kinetic energy determined by the potential energy of their repulsion at that distance. Therefore, observing the process at different internuclear distances changes the kinetic energy available to be shared by the outgoing electrons. Could the recently observed changes in the ejection pattern of the electrons with internuclear distance be due to this purely kinematic effect, or do they really reflect changes in electron correlation? This question could not be answered by the experiment because it was performed with a single-photon energy. Precise solutions of the Schrödinger equation are required to unambiguously resolve this issue and thereby test the assertion that large changes in the ejection pattern are the signature of changes in electron correlation with bond distance.

The calculation we report here required a major extension, described in the supporting online material (*9*), of the methods that only recently solved such problems for isolated atoms (*6*, *10*, *11*). Only the first steps toward a precise solution of the molecular problem, based on a numerical solution of the full Schrödinger equation with no appeal to models, had previously been taken with the use of any methods (*12*, *13*), and those efforts produced only the total ionization probability and not the angular patterns of the ejected electrons. The only assumption we have made here is that the nuclei do not move during the time required to eject the two electrons. This simplification is consistent with the familiar Born-Oppenheimer approximation, which is valid for electrons moving much faster than the nuclei, as is the case in the current experiments on D_2 and for the same process in H_2.

One of the clearest molecular effects that can be observed both by experiment and by our current theory is manifested in the angular pattern of two ejected electrons or triple differential cross section (TDCS), so called because it depends on the directions of the two ejected electrons and their energy sharing. We have calculated the TDCS for double photoionization of the helium atom at 24.5 eV excess energy (Fig. 1A) using the methods we

[1]Department of Computer Science, K.U. Leuven, Celestijnenlaan 200A, B-3001 Heverlee, Belgium. [2]Departamento de Química C-9, Universidad Autónoma de Madrid, 28049 Madrid, Spain. [3]Lawrence Berkeley National Laboratory, Chemical Sciences, Berkeley, CA 94720, USA. [4]Department of Applied Science and Department of Chemistry, University of California, Davis, CA 95616, USA.

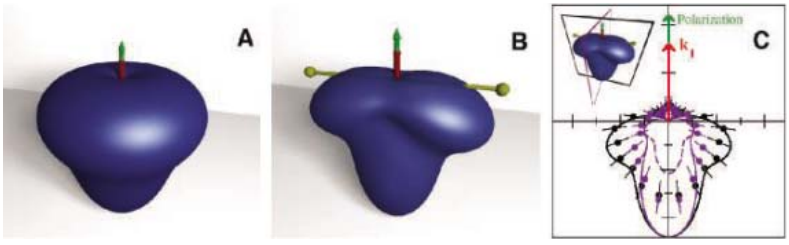

Fig. 1. Angular distribution (TDCS) of ejected electrons with direction of one electron (red arrow, momentum k_1) with 90% of the available energy fixed along the polarization direction (green arrow). A manifestation of a purely molecular effect can be seen by comparing the angular distributions (TDCS) for (**A**) helium atom with (**B**) H_2 molecule with axis oriented perpendicular to the polarization. (**C**) Comparison with relative experimental measurements shows that two cuts of the distribution in (B) have been observed to verify this effect. The dashed curve in (C) shows how the TDCS changes when the fixed electron has 80% of the available energy.

developed earlier for atomic problems (*10*). We chose 24.5 eV excess energy to facilitate comparison with the results for molecular hydrogen. For the case shown, one electron goes out along the direction of the polarization vector of the incident photon with 90% of the available energy, and the cross section is plotted as a function of the direction of the other electron. The resulting distribution for an atom must have cylindrical symmetry about the common axis of the polarization and the direction of the fixed electron. In contrast, when the H_2 molecule, oriented perpendicular to the polarization direction, is doubly ionized with the same excess energy, our calculations show that the cylindrical geometry of the atomic case (Fig. 1A) becomes flattened along the direction of the molecular axis (Fig. 1B).

This molecular effect has been observed experimentally (Fig. 1C) (*14*, *15*). The experimental data were collected in a manner that corresponds to making two cuts of the three-dimensional distribution shown in Fig. 1B, and they reveal a widening of the distribution when the cut is parallel to the molecule. The detailed comparison of theory with the existing experiments is complicated by the finite resolution of the experiment in both angle and energy. The experiment accepts all events with "fixed" electron angles ±20°, "swept" electron angles ±17.5°, molecular angles ±25°, "fixed" electron energies ≥80% of that available, and a range of proton kinetic energies of ~1 eV. The calculated data in Fig. 1C also give some indication of the major changes that occur in the TDCS with relatively small changes in the energy sharing between the electrons. To determine the full implications of these experiments, we focused on the accurate theoretical description of this process without the complication of averaging over the experimental resolutions.

To see the effect on the angular dependence of varying the internuclear distance, we must choose an experimental geometry that is sensitive to that variation. As the molecule rotates between being parallel and perpendicular to the polarization direction, the dipole-allowed transitions between the initial state of $^1\Sigma_g$ symmetry and final continua of $^1\Sigma_u$ (parallel) and $^1\Pi_u$ (perpendicular) symmetries contribute in varying proportion to the observed TDCS. The behavior of the TDCS for an angle between the molecule and polarization vector of 15° (Fig. 2) probes both those components visibly. Experimental data resolving the orientation of the molecule sufficiently to show the smaller $^1\Sigma_u$ component [a factor of 10 smaller (*12*, *13*) than $^1\Pi_u$] unambiguously have yet to be published. In the existing experiments (*5*, *14*, *15*), differences in the observed cross section for various internuclear distances, with one electron ejected perpendicular to the plane formed by the molecular axis and polarization vector, were attributed to changes in the electronic correlation of the initial state of the D_2 molecule (*5*).

To explore that effect uncomplicated by the finite resolutions of the experiment, we examined the TDCS at three internuclear distances: the equilibrium position of the nuclei and the inner and outer classical turning points of their vibrational motion in the ground vibrational state. The behavior of the TDCS (Fig. 2A) shows a marked variation with internuclear separation that is particularly notable when seen in complete three-dimensional views of the angular dependence of the second electron for a fixed ejection direction of the first. The differences are even larger than the experiment was able to reveal because of its finite angular and energy resolutions.

However, the variation shown in Fig. 2A does not establish that the effect on the cross section of varying internuclear distance is a result of changes in electron correlation. The electrons liberated by ionization at the inner vibrational turning point share 5.42 eV less energy than electrons released by ionization at the outer turning point, because the nuclei carry off different amounts of repulsion energy. The experiments (*5*, *14*, *15*) were performed at a photon energy 24.5 eV above the threshold for double ionization of D_2 in its ground vibrational state. This variation with internuclear distance of kinetic energy shared by the outgoing electrons is more than 20% of that nominal value. Double photoionization cross sections of atoms are known to be sensitive to energy differences and very sensitive to changes in energy sharing. Therefore, one must suspect that changes observed in the molecular TDCS with varying internuclear distances might only be due to the change in the effective energy transmitted to the outgoing electrons.

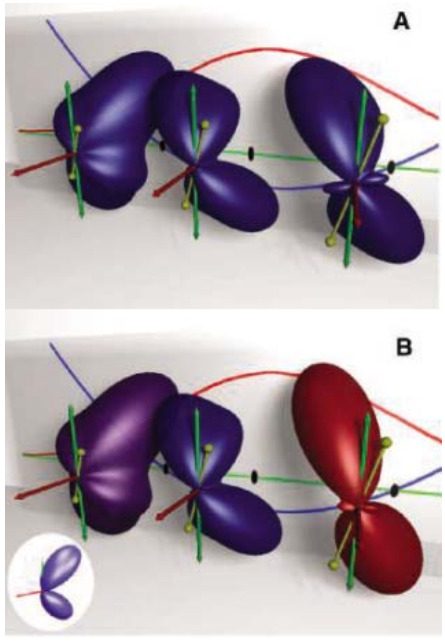

Fig. 2. Effect on the angular distribution for D_2 of varying the internuclear distance. (**A**) TDCS is shown with molecular axis (yellow) 15° from polarization vector (green arrow) and fixed electron (red arrow) leaving perpendicular to that plane with 50% of the available ejection energy. (Left) Inner vibrational turning point; (middle) equilibrium internuclear distance; (right) outer turning point. Background shows ground state potential curve (blue), ground state vibrational wave function (red), and energy (green line, with black dots indicating equilibrium and inner and outer turning points). (**B**) The effect on the angular distribution of varying internuclear distance is primarily due to changes in molecular electronic correlation. The angular distributions closely resemble those in (A) when the photon energy is varied, so as to produce the same amount of final kinetic energy to be shared by the outgoing electrons, regardless of the internuclear distance at which ionization occurs. Inset shows the corresponding atomic case (helium).

To investigate that question, we examined the TDCS for the same three internuclear distances but now calculated with different photon energies so that the outgoing electrons always shared 24.5 eV of energy (Fig. 2B). The resulting variations in the cross section with varying internuclear distance were both qualitatively and quantitatively similar to those observed when a single-photon energy was used (Fig. 2A). This comparison therefore establishes that experiments observing the complete dynamics of the breakup process can measure changes in the effect of electron cor-

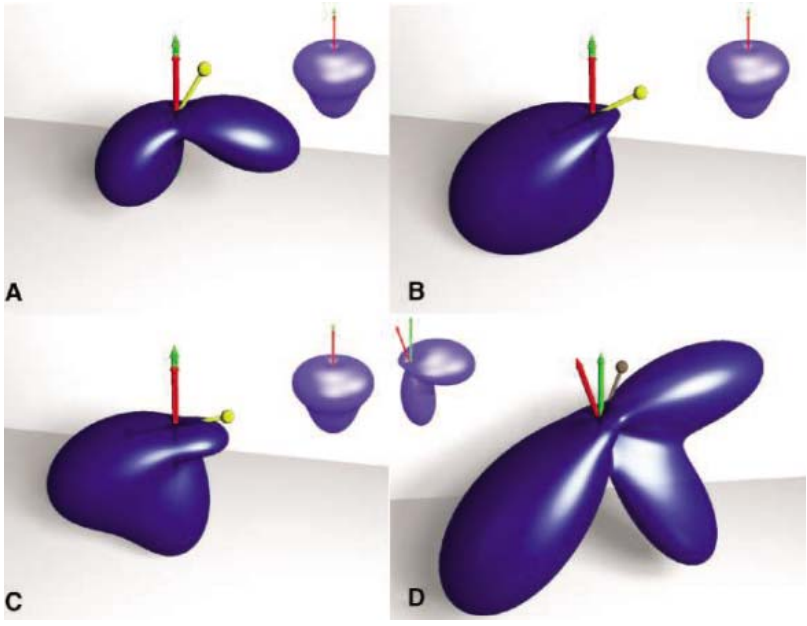

Fig. 3. Effects of molecular orientation on the angular distribution of ejected electrons. For the fixed electron ejected along the polarization direction with 90% of the kinetic energy, the molecule makes an angle with the polarization of (**A**) 30°, (**B**) 60°, and (**C**) 75°, and splits the corresponding pattern for the helium atom (insets) into two lobes which vary in size and ultimately show a tendency to align along the molecular axis as in Fig. 1B. The cross section in (A) is about one-fourth the magnitude of (B) and (C). (**D**) A case in which the molecule and fixed electron have 10% of the kinetic energy, both at 20° from the polarization vector but on opposite sides, yielding an ejection pattern markedly different from the corresponding atomic one.

relation that are purely molecular in character and vary with molecular geometry.

The three-dimensional representations of the TDCS seen in Figs. 1 and 2 can be viewed as variations on a theme already seen in the helium atom. There, the angular distribution of the plotted electron shows an apparent tendency to go out in the direction more or less opposite to that of the fixed electron (*10*). This pattern is modified in the case of equal energy sharing by selection rules (*16*–*18*) that place a zero in the angular distribution for back-to-back ejection of the electrons. The molecular cases in Fig. 2 show interesting modifications of the resulting simple two-lobed pattern seen in the case of the helium atom.

To see how the presence of the nuclei in the molecular case modifies the angular distributions, we can examine how the TDCS changes when the orientation of the molecule is varied while keeping the direction of the fixed electron unchanged. Choosing the fixed electron along the polarization vector and with 90% of the available kinetic energy (as in Fig. 1B), we compared the atomic distribution with that obtained for the molecule when it makes an angle of 30° (Fig. 3A), 60° (Fig. 3B), 75° (Fig. 3C), or 90° (Fig. 1B) with the polarization vector. This sequence suggests the effect of nuclear attraction on the slower outgoing electron for a set of cases for which no simple symmetry selection rules apply. At 30° the nuclei break the single lobe of the atomic pattern into two lobes, which change to have very different sizes by 60°. The overall flattening of the distribution in the direction of the molecular axis is evident in all four figures.

A comparison of the angular distribution at 75° (Fig. 3C) with the more symmetric 90° pattern (Fig. 1B) shows how attraction to the nuclei tends to orient some parts of the ejection pattern along that axis, but the set of four patterns shows that this effect is strongly modified by the forces of electron repulsion and the applied radiation field. At some geometries, such as the one shown in Fig. 3D, the presence of the nuclei alter the corresponding atomic ejection pattern even more. In this case, the two lobes of the atomic angular distribution are broken into three lobes, and no simple model appears to explain the resulting TDCS.

This initial view of accurate calculations of the angular dependence of double photoionization of a molecule shows that experiments measuring the TDCS for different internuclear distances can reveal the signature of essentially molecular effects in electronic correlation. The wealth of information that can only be obtained from calculations and experiments on oriented molecules is highlighted by the fact that, when averaged over molecular orientations, the TDCS patterns for molecular hydrogen are almost identical to those for helium (*19*). Detailed comparison of measurements on oriented molecules with accurate theory may form the basis for improving existing models (*20*–*22*) to show clearly how simple physical effects are manifested in the TDCS. However, some aspects of the angular distributions are the signatures of the essentially complicated physics of electron correlation and may not be easy to model. Nonetheless, the present calculations indicate that there is promise that such analyses can be performed for double photoionization of other molecules to probe correlation in more complicated molecular cases. We expect that this class of experiment and theory will be used, for example, to study the difference between the correlation effects that dominate when two electrons are ionized from the same molecular orbital in a molecule like CO, as opposed to when those electrons are ionized from different shells leaving behind a different electronic state of the CO^{++} ion.

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Real-Time Observation of Molecular Motion on a Surface

Ellen H. G. Backus,[1] Andreas Eichler,[2]
Aart W. Kleyn,[1,3] Mischa Bonn[1,4]

The laser-induced movement of CO molecules over a platinum surface was followed in real time by means of ultrafast vibrational spectroscopy. Because the CO molecules bound on different surface sites exhibit different C–O stretch vibrational frequencies, the site-to-site hopping, triggered by excitation with a laser pulse, can be determined from subpicosecond changes in the vibrational spectra. The unexpectedly fast motion—characterized by a 500-femtosecond time constant—reveals that a rotational motion of the CO molecules, rather than pure translation, is required for this diffusion process. This conclusion is corroborated by density functional theory calculations.

The motion of molecules from one adsorption site to another is the most elementary process of bond-breaking and-making on a surface and is a key step in processes such as catalysis and crystal growth. A fundamental understanding of surface molecular motion requires insights on both the molecular length and time scales, necessitating subnanometer spatial resolution and subpicosecond temporal resolution.

Real-space observations of site-to-site surface molecular motion are readily obtained by scanning tunneling microscopy (STM) (*1*). Recent STM experiments have even demonstrated control over molecular motion on a surface, induced by electrons from the STM tip (*2*–*5*) or by a femtosecond laser (*6*). These studies have provided detailed insights into the energetics, local directionality, and site dependence of molecular surface motion.

In contrast, the direct real-time observation of surface molecular motion has not been reported to date, although such data would provide important insights into the dynamics and molecular mechanism behind surface molecular motion. Indeed, a recent time-resolved study by Stépán *et al*. (*7*) and combined laser-STM experiments by Bartels *et al*. (*6*) have revealed detailed quantitative information on surface motion induced by an ultrashort laser pulse, as also shown previously for other surface reactions (*8*–*12*). However, in these studies the time coordinate was probed indirectly, by monitoring the equilibrated system after the induction of molecular motion with a laser pulse.

The direct time-resolved study of surface processes would allow the observation of energy flow between the substrate and the vibrational and rotational modes of the adsorbed molecule, and elucidate how these modes are coupled to the reaction coordinate (such as diffusion). Such information would unambiguously reveal the detailed molecular mechanism and intrinsic rates of surface reactions.

Here we report the direct real-time observation of surface molecular motion by means of time-resolved vibrational spectroscopy to provide real-time "snapshots" of the process. We observed subpicosecond hopping of CO molecules from step sites to terrace sites. Both the experiments and density functional theory (DFT) calculations reveal that, in contrast to the common belief that lateral motion is preceded by excitation of only the translational mode, excitation of the rotational mode of the molecules is a crucial step in the hopping process.

Our approach relies on CO molecules having a different internal C–O stretch vibrational frequency when adsorbed on the different sites of a stepped Pt(533) surface (Fig. 1A) (*13*), which provides indirect subnanometer spatial resolution along the coordinate perpendicular to the steps, as shown previously (*14*, *15*). The Pt surface consisted of 7 Å–wide flat terraces separated by monatomic steps. The C–O stretch vibrations of CO on steps and terraces monitored with femtosecond sum-frequency generation (SFG) (*16*) differed by ~20 cm^{-1} (Fig. 1B), so that CO molecules on the two sites were readily distinguished.

To obtain a surface with preferential occupation of the step sites, we made use of the stronger binding of CO molecules on steps (~2 eV) as compared to terrace sites (~1.5 eV) for isolated molecules (*13*, *17*). Brief heating of a fully CO-saturated surface to 420 K preferentially removed CO molecules from the terrace sites; CO molecules at the steps desorbed at a higher temperature. The heating was terminated when the SFG spectrum (Fig. 1B, black line) revealed only CO on step sites at 2080 cm^{-1} (*13*). The spectrum for a CO-saturated surface, where the majority of CO is adsorbed on terrace sites, shows a resonance at 2100 cm^{-1} (*13*), which is the vibrational frequency of CO on terraces. The CO molecules bound to the step sites are invisible because of the effects of dipole-dipole coupling: The vibrational intensity from the oscillating CO dipoles on the step sites is transferred to the

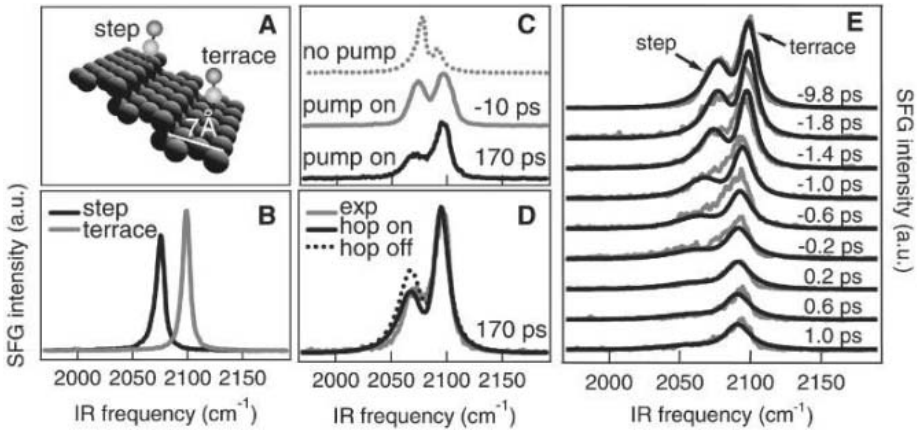

Fig. 1. (**A**) A schematic drawing of CO bound on top of step and terrace sites of the Pt(533) surface, consisting of four-atom-wide (111) terraces and monatomic (100) steps. (**B**) Sum-frequency spectra for CO bound on the step and terrace sites, exhibiting resonances at 2080 and 2100 cm^{-1}, respectively. a.u., arbitrary units. (**C**) SFG spectra without pump (gray dotted line) and before (–10 ps, gray solid line) and after (170 ps, black solid line) excitation of a Pt(533) surface partially covered with CO at 100 K with a 130-fs laser pulse at a fluence of 60 J m^{-2}. After 170 ps, the terrace peak has increased in intensity, whereas the step peak is reduced by a factor of 2: Molecules have moved from step to terrace sites. (**D**) The normalized experimental (exp) SFG spectrum at 170 ps (gray) of (C), together with the normalized calculated spectra (black). The black solid and dashed lines show calculated spectra with and without motion of 10% of the CO molecules of the step sites, respectively. (**E**) Experimental (gray) and calculated (black) pump-probe spectra at short delay times for a surface partially covered with CO (75% of steps and 20% of terrace sites occupied) for excitation with a laser pulse with a fluence of 60 J m^{-2}.

[1]Leiden Institute of Chemistry, Leiden University, Post Office Box 9502, 2300 RA Leiden, Netherlands. [2]Institut für Materialphysik and Center for Computational Materials Science, Universität Wien, Sensengasse 8/12, A-1090 Wien, Austria. [3]Stichting voor Fundamenteel Onderzoek der Materie (FOM) Institute for Plasma Physics Rijnhuizen, Post Office Box 1207, 3430 BE Nieuwegein, Netherlands. [4]FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, Netherlands.

more quickly oscillating COs (at higher frequency) on the terrace sites (*18*).

In our experiment, we induced the energetically uphill motion of CO molecules from step to terrace sites by exciting the substrate with an ultrashort laser pulse (fluence, 60 J m^{-2}). To achieve maximum sensitivity for the CO hopping process, we started with occupancies of ~100% of the steps and ~10% of the terrace sites, the spectrum for which is shown as the dotted line in Fig. 1C. Estimates of the occupation of the step and terrace sites were obtained from an uptake curve and the dipole-dipole coupling model (*18*). Because desorption [binding energy (E_{bind}) = 1.5 to 2 eV] by laser-induced heating is readily achieved (*19*), and the activation energy for diffusion is typically a fraction of E_{bind}, we expect diffusion to be triggered at lower fluences. Indeed, pump excitation of the Pt surface with CO preferentially at the step sites resulted in a steady-state situation, with CO more evenly distributed on step and terrace sites, as is apparent from comparing the gray curves (pump on/pump off) in Fig. 1C. After excitation, the terrace peak is even larger than the step peak, because the terrace CO (~15% occupation of the terrace sites) efficiently borrows vibrational intensity from the step CO (~90% occupation of the step sites) (*18*). Thus, the system is extremely sensitive to the motion of CO from steps to terraces.

The CO molecules can clearly undergo laser-induced movement from step to terrace sites. We also find that blocking the pump laser allows the molecules to diffuse back to the step sites in ~10 s, in agreement with (*20*). If the motion is induced at an initial surface temperature of 400 K rather than 100 K, repopulation of the step sites occurs on ~100-ps time scales because of thermal diffusion; that is, ~10^{11} times faster than at 100 K, which is consistent with the diffusion coefficients reported in (*21*).

The fraction of molecules that moves from step to terrace sites in a single laser shot can be determined by comparing pump-probe spectra directly before and after the excitation pulse. Figure 1C depicts spectra 10 ps before the pump pulse (negative delay, under steady-state conditions with ~90% occupation of the step sites and ~15% occupation of the terrace sites) and 170 ps after excitation. At 170 ps, the terrace peak has gained intensity and the step peak is reduced by a factor of 2. Taking into account that, at 170 ps, the system is still at slightly elevated temperature, we can reproduce the spectrum using the dipole-dipole coupling model (*18*), with 10^{+10}_{-5}% of the CO molecules on the steps hopping to terrace sites. The calculated curve (*18*) is shown as the solid curve in Fig. 1D. Hopping of 10% of the step CO molecules to the terrace sites results in a disproportionately large intensity decrease of 20% of the step peak (compare dotted and solid lines in Fig. 1D) because of dipole-dipole coupling effects. The observed intensity change cannot be explained by desorption, because at the fluence used, less than 0.1% of the molecules desorbs in a single shot. Also, the time scale of signal recovery is not consistent with readsorption of CO from the background or the relaxation of laser-induced changes in the orientation of molecules. Diffusion to or from bridge sites can be excluded as well, because no change is observed in the bridge peaks for step and terrace around 1900 cm^{-1} (*13*).

The dynamics of the motion of the CO molecules from step to terrace sites is obtained from pump-probe spectra near zero delay, shown in Fig. 1E for steady-state occupation of 75 and 20% of the step and terrace sites, respectively. It is evident that the ratio of step and terrace peaks changes on ultrafast time scales: The step peak disappears almost completely within a picosecond, indicating that the motion from step to terrace sites occurs on this time scale. The observation that the effect of the pump pulse appears in the spectra already at negative delay can be explained by the perturbation of the free-induction decay (*22*, *23*).

The details of the mechanism and rate of the laser-induced diffusion process are contained in the time-dependent fraction of the step molecules. This information was extracted from the data by a full spectral simulation. The spectral changes are caused by two effects: (i) the change of the occupation of the different sites due to motion of CO molecules from step to terrace sites and (ii) the effects of the laser-induced heating of the system on the internal C–O stretch vibration. The latter was determined in independent measurements at a lower fluence at which no laser-induced motion occurs. Laser excitation results in a transient redshift, line broadening, and decrease in intensity of the C–O stretch vibration for CO on both step and terrace sites, even when no motion occurs (Fig. 2). This laser excitation of the Pt substrate still creates a hot electron gas, from which energy transfer to the CO molecules can occur, resulting in the excitation of the three CO low-frequency modes: (i) the frustrated translation, (ii) the frustrated rotation, and (iii) the Pt–CO stretch. The degree of electron-induced excitation of each mode can be characterized by a time-dependent temperature (T_{ads}). The internal C–O stretch is not itself excited, because its frequency is too high, but it is affected (Fig. 2) by excitation of the low-frequency [ν = 35 cm^{-1} (*19*)] frustrated translation mode through anharmonic coupling (*22*, *23*). The relation between this excitation (expressed in T_{ads}) and the internal C–O stretch frequency can be obtained from temperature-dependent measurements at thermal equilibrium (*24*).

This sequence of events—excitation of the substrate electrons, energy transfer to mode (i) of the CO molecules, and the associated changes in the C–O stretch vibration—can be described with a friction model (*25*), the result of which is shown as black lines in Fig. 2. The key parameter of this model is the time scale on which energy flows between the electrons of the Pt and the frustrated translational mode (i), τ_{el}. To reproduce the data in Fig. 2, coupling times of τ_{el} = 2.5 ± 0.5 ps and 4 ± 0.5 ps for terrace and step, respectively, are required. The shortcoming of the model in reproducing the intensity for small negative delay originates from an increase of the Raman tensor due to changes in the electronic structure of the Pt substrate immediately after laser excitation, as verified in an independent measurement without CO. However, width and central frequency are well reproduced.

These coupling times can now, in combination with the dipole-dipole coupling model, be used to calculate the SFG spectra of Fig. 1E, with inclusion of the hopping process. To reproduce the dynamic behavior of the terrace peak, the magnitude of its peak shift and line width change have to be divided by 2, presumably because of the appearance of additional terrace CO molecules during the hopping process. The time scale of the peak shift and line width is unchanged, however. In the

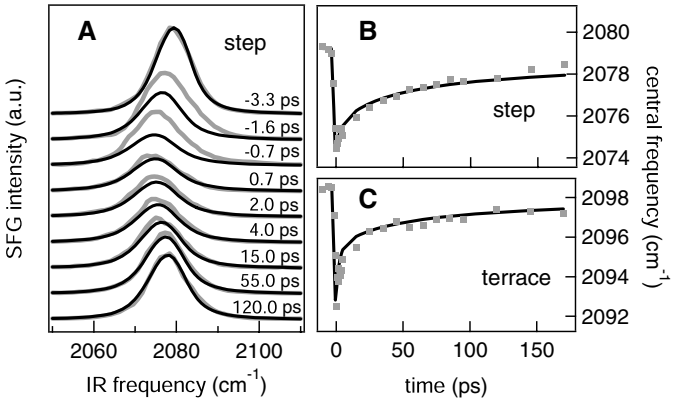

Fig. 2. (**A**) Experimental (gray) and calculated (black) time-resolved sum-frequency spectra for CO on Pt(533), with occupation of the step sites at 100 K. At time zero, a 130-fs pump pulse with a fluence of 11 J m^{-2} excites the Pt substrate. (**B** and **C**) Central frequency obtained by fitting a Lorentzian line shape to the experimental (gray dots) and calculated (black lines) SFG spectra (A) for CO on the step (B) and terrace (C) sites, the latter with a fluence of 16 J m^{-2}.

modeling, the time-dependent hopping probability from the step site to the terrace site is approximated by a function that is formed by a Gaussian rising edge, followed by an exponential decay (*26*). The integral of this function gives the time-dependent step site occupation.

The calculations confirm quantitatively that, in order to reproduce the data at short delays (Fig. 1E), a subpicosecond change of the site occupation is required, as is already evident from the raw data. The resulting step site occupation is plotted in Fig. 3A. The shape of the curve of Fig. 3A is very sensitive to the spectra at small positive delay. The time-dependent calculated SFG spectra are plotted in Fig. 1E and are in good agreement with the experimental data. As is the case in Fig. 2A, the calculated intensity is slightly too low at small negative delays. The exponential time obtained from the calculation is 500 ± 150 fs.

It has been generally assumed that molecular diffusion of diatomic adsorbates is controlled by excitation of the frustrated translation (inset in Fig. 3) (*4*, *5*, *7*). Although this model is intuitively appealing, the actual reaction coordinate may involve a more complex combination of modes. We calculated the time-dependent hopping probability expected for motion controlled by the frustrated translational mode. Using the τ_{el} = 4 ps coupling time derived from Fig. 2 for CO on steps, we can directly calculate the time-dependent temperature associated with the frustrated translational mode (T_{ads}) (Fig. 3B) and calculate the hopping probability according to an Arrhenius-type expression $P_{hop}(t) = \theta(t)k_0\exp\{-E_a/[k_B T_{ads}(t)]\}$, given the activation energy (E_a) of 0.4 eV (*21*) and the prefactor (k_0) of 10^{12} s^{-1} (*20*).

Remarkably, the agreement between the calculated result and the experimentally observed hopping probability (Fig. 3C, obtained by differentiating the hop fraction of Fig. 3A) is very poor, despite the independent calibration of the temperature of the frustrated translational mode with SFG (Fig. 2). However, quantitative agreement between model and data is obtained for a hopping process controlled by excitation of the frustrated rotational mode [mode (ii), ν = 411 cm^{-1} (*24*), inset in Fig. 3]. This mode (see Fig. 3B for its time-dependent temperature) is coupled to the hot electrons in the Pt, with τ_{el} = 0.1 ps as established in (*19*) and also in agreement with the coupling time previously reported for this mode on Pt(111) (*23*). Because of this very efficient coupling, hopping can occur on the observed fast time scale. Not only was the temporal evolution reproduced, but also the calculated total hop fraction of ~10% is in good agreement with our experimentally observed 10^{+10}_{-5}%. No additional coupling to the frustrated translation is required to account for our experimental data (*27*). Thus, a rotational motion rather than a translational motion is essential for the hopping process of CO molecules from step to terrace sites.

These experimental observations are corroborated by DFT calculations (*17*), which reveal that the barrier for motion onto the terrace next to the step (~0.4 eV) is substantially lower than that for motion onto the terrace down the step (~0.6 eV); the majority of the hopping CO molecules will migrate onto the upper terrace. The calculated value of the diffusion barrier is in good agreement with the 0.4 eV observed experimentally for CO on a stepped Pt surface (*21*). The reaction pathway for the motion of CO onto the upper terrace is depicted in Fig. 4. From the initial (is) and intermediate (im) states, one can see that the CO molecules moving onto the upper terrace first perform a frustrated translational motion: The C and O atoms move in the same direction. This is a joint motion around the center of the underlying Pt atom (*28*), not parallel to the surface. At the first transition state (ts1), however, a rotational motion of the molecule, with the C and O atoms moving in opposite directions, compensates for this tilting, so that the reaction intermediate (ri), with CO bound in a bridge configuration, can be reached. To reach the final state (fs), a similar motion has to be performed. The entire process thus requires excitation of both the frustrated translation and frustrated rotation, but the former (frequency, 35 cm^{-1}) is thermally excited at 100 K, effectively resulting in a precursor state.

Hence, excitation of the frustrated rotation is pivotal for CO hopping, in agreement with our experimental observations. For diffusion on a flat surface as well, most likely the frustrated rotation is crucial. Because of the atomic corrugation of the surface, the frustrated translational mode always involves rotation of the molecular axis with respect to the surface normal. This rotation has to be compensated for in order for the molecule to settle on the neighboring site, which can only be achieved by excitation of the frustrated rotational mode. Our findings illustrate the intricacies of mode coupling at surfaces: Contrary to common belief, the frustrated rotational mode is strongly coupled to the

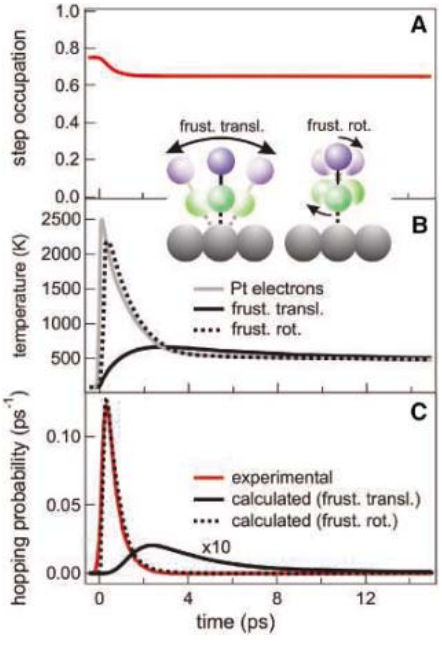

Fig. 3. (**A**) Occupation of the step sites as a function of time after the pump pulse with a fluence of 60 J m^{-2}, obtained by fitting the spectra of Fig. 1E, assuming exponential hopping dynamics and a Gaussian response function. (See text for details about the fitting procedure.) (**B**) Time-dependent electron temperature after excitation at time = 0, with a pump pulse with an adsorbed fluence of 60 J m^{-2}, together with the temperatures of the frustrated translational (electronic coupling time, 4 ps) and the frustrated rotational (electronic coupling time, 0.1 ps) modes. (**C**) Experimental and calculated hopping probability as a function of time. The experimental curve was obtained by differentiating the step occupation of (A). Calculations were performed for hopping due to excitation of the frustrated translation and rotation modes, using the time-dependent adsorbate temperature of (B). Clearly, coupling along the frustrated rotation is in agreement with the experimental results: The shape and intensity are very well reproduced (note that the calculation is not scaled to fit). The inset depicts the molecular motion associated with the two modes.

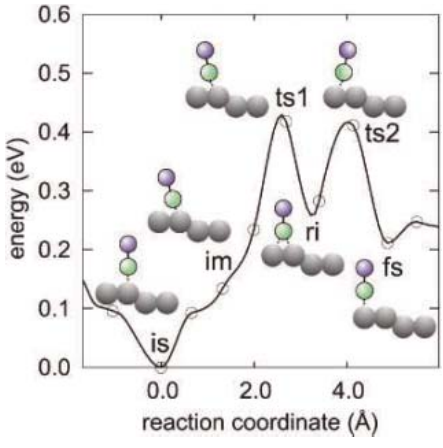

Fig. 4. Reaction pathways for the diffusion of CO from the step sites to the upper terrace, obtained with DFT calculations (calculated for the situation in which 75% of all step sites are initially occupied.). To go from the initial state (is) to the final state (fs), the CO molecule must pass two transition states (ts1 and ts2) and a reaction intermediate (ri). Whereas initially the motion is dominated by the frustrated translation, the molecule has to perform a rotational motion as well to overcome ts1. After passing ts1, the molecule arrives in the reaction intermediate (ri), consisting of a bridge state. Before reaching fs, CO bound atop the terrace site, the molecule again performs a translational and rotational motion. Experimentally, it has been concluded that there is a significant tilt angle away from the surface normal for CO on step sites (*29*). Nevertheless, even for tilted molecules the crucial step over the transition state is still the frustrated rotation: To reach the final position, the Pt–C bond has to be broken and reformed in the new position on the terrace, requiring a rotation of the molecule. An initial change in the tilt angle can be achieved by a translation motion.

coordinate for diffusion, and, in the case of our experiment, dominates the diffusion away from step sites.

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16. The experiments were performed in an ultrahigh vacuum chamber with a base pressure of 2×10^{-11} mbar, in combination with a Ti:sapphire femtosecond laser system (Quantronix, Titan). For the femtosecond pump-probe SFG experiments, three laser pulses were used to induce the motion of CO molecules: the SFG probe pair [a combination of a weak infrared (IR) and weak visible beam] and an intense visible pump beam (~130 fs). Both the IR pulses [2080 cm^{-1}, full width at half maximum (FWHM) = 200 cm^{-1}, 6.5 mJ], generated by a traveling-wave optical parametric amplifier of superfluorescence, and the visible upconversion pulses (12550 cm^{-1}, FWHM = 8 cm^{-1}, 4.5 mJ) were p-polarized and focused onto the surface by the same parabolic mirror (f = 100 mm). 1.3 mJ of the 800-nm laser output was used as a pump pulse (p-polarized). The beam diameter of the pump beam was 1 mm, which is approximately four times larger than the diameter of the probe beams. The time resolution was better than 200 fs. The SFG signal was detected with a spectrometer and a charge-coupled device camera. To minimize steady-state heating effects of the substrate, the laser frequency was reduced to 83 Hz by a chopper. Unless otherwise noted, the experiments were performed at a surface temperature of 100 K. For the highest-excitation fluences, very small amounts (<1 per mil per shot) of CO, dosed via background dosing at 100 K, were desorbing. Therefore, the experiments at fluences exceeding 50 J m^{-2} were performed with a CO background pressure of $>1 \times 10^{-8}$ mbar to keep a constant surface coverage.
17. Structures and binding energies were calculated with the Vienna Ab-initio Simulation Package (VASP) (see http://cms.mpi.univie.ac.at/vasp/). VASP is a plane wave–based density functional code employing the projector augmented wave method (*30*). The surface was modeled by a 19-layer slab in a 4×1 cell containing four atoms per layer, sampled by a grid of $(3 \times 2 \times 1)$ k points. A cutoff energy for the expansion of the plane waves of 400 eV was found to be sufficient for an accurate description. For exchange and correlation contributions, generalized gradient corrections according to Perdew *et al.* (*31*) were applied. Pathways and activation energies for diffusion of CO were determined with the nudged elastic band method (*32*).
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26. The width of the Gaussian determines the rise time of the signal and corresponds to the rate of adsorbate heating; the exponential decay is caused by adsorbate cooling. Both are determined by the coupling time. Because cooling is the slowest process, the exponential time scale is the largest of the two, and thus provides an upper limit for the time scale on which adsorbate motion occurs.
27. Coupling to the frustrated rotation is not observed in the SFG spectra of Fig. 1, because the anharmonic coupling between the frustrated rotation and the internal C–O stretch vibration is apparently too small. The data at low fluence (11 J m^{-2}) can be described with only anharmonic coupling to the frustrated translation without invoking any effect of the frustrated rotation. This allows us to determine an upper limit for the anharmonic coupling between the frustrated rotation and the internal C–O stretch vibration. Based on the known values for the frequencies of the different modes and the anharmonic coupling for the frustrated translation (*24*), the absolute value for the coupling of the frustrated rotation is less than 1 cm^{-1}. This will result in an additional shift to the spectra in Fig. 1E of at most 2.5 cm^{-1}.
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Multistep Synthesis of a Radiolabeled Imaging Probe Using Integrated Microfluidics

Chung-Cheng Lee,[1]* Guodong Sui,[3,4]* Arkadij Elizarov,[2]* Chengyi Jenny Shu,[5] Young-Shik Shin,[2] Alek N. Dooley,[6] Jiang Huang,[8] Antoine Daridon,[8] Paul Wyatt,[8] David Stout,[4] Hartmuth C. Kolb,[3,9] Owen N. Witte,[3,5,7] Nagichettiar Satyamurthy,[3] James R. Heath,[2,3,4] Michael E. Phelps,[3,4] Stephen R. Quake,[1,10]† Hsian-Rong Tseng[3,4]†

Microreactor technology has shown potential for optimizing synthetic efficiency, particularly in preparing sensitive compounds. We achieved the synthesis of an [^{18}F]fluoride-radiolabeled molecular imaging probe, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), in an integrated microfluidic device. Five sequential processes—[^{18}F]fluoride concentration, water evaporation, radiofluorination, solvent exchange, and hydrolytic deprotection—proceeded with high radiochemical yield and purity and with shorter synthesis time relative to conventional automated synthesis. Multiple doses of [^{18}F]FDG for positron emission tomography imaging studies in mice were prepared. These results, which constitute a proof of principle for automated multistep syntheses at the nanogram to microgram scale, could be generalized to a range of radiolabeled substrates.

Continuous-flow microreactors (*1*–*3*) have recently been used to manipulate individual chemical processes on nanoliter to microliter scales. The advantages of such chemical reaction circuits include enhanced heat transfer performance, faster diffusion times and reaction kinetics, and improved reaction product selectivity (*4*–*6*). For example, in microfluidic environments, triphasic hydrogenation (*7*) can be achieved with higher reaction efficiency, the inorganic synthesis of high-quality CdSe nanocrystals has been demonstrated (*8*), and chemical processes involving highly reactive intermediates can be executed with superior reaction selectivity (*9*). However, challenges remain in applying the technology to sequential syntheses of fine chemicals and pharmaceuticals.

In multistep procedures, flow-through systems are plagued by cross-contamination of reagents from different steps; side reactions and poor overall yield result from the inability

[1]Department of Bioengineering, [2]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA. [3]Department of Molecular and Medical Pharmacology, [4]Crump Institute for Molecular Imaging, [5]Department of Microbiology, Immunology, and Molecular Genetics, [6]UCLA Mass Spectrometry Facility, [7]Howard Hughes Medical Institute, University of California, Los Angeles, CA 90095, USA. [8]Fluidigm Corporation, 7100 Shoreline Court, South San Francisco, CA 94080, USA. [9]Molecular Imaging, Siemens Medical Solutions USA Inc., 6140 Bristol Parkway, Culver City, CA 90230, USA. [10]Department of Bioengineering, Stanford University, Stanford, CA 94305, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: quake@stanford.edu (S.R.Q.); hrtseng@mednet.ucla.edu (H.-R.T.)

to confine each individual step. Microfluidic batch devices with integrated microvalves show promise for the automation of multiple, parallel, and/or sequential chemical processes on a single chip under digital control. By analogy, this technology has already been successfully applied to biological problems (*10*).

A compelling application is in the preparation of organic compounds bearing short-lived isotopes whose emission permits detailed mapping of biological processes in living organs. In conjunction with positron emission tomography (PET) (*11*), the development of sensitive radiolabeled molecular probes is crucial for expanding the capability of target-specific in vivo imaging for biological research, drug discovery, and molecular diagnostics. The United States already has a vast network of PET cyclotron production sites in place as convenient sources for radiolabeled precursors [e.g., [^{18}F]fluoride, [^{11}C]CO_2, and [^{11}C]methyl iodide (MeI)] and a few labeled biomarkers. The capacity for diversifying radiolabeled probe structure is therefore limited only by the cost, speed, and efficiency of synthetic methods. A central challenge in this regard is the half-life of the radiolabels.

The synthesis (*12*) of the [^{18}F]-labeled molecular imaging probe 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) in an integrated microfluidic chip was chosen as a proof-of-principle study. This compound is the most widely used radiolabeled molecular probe, with more than 1 million doses for patient diagnosis produced in the United States in 2004 and a similar number in the rest of the world (*13*). The brief half-life of [^{18}F]fluorine ($t_{1/2}$ = 110 min) makes rapid synthesis of doses essential. Today, [^{18}F]FDG is routinely produced in about 50 min with the use of commercial synthesizers (*14*), which are expensive (~$140,000) and produce ~10 to 100 doses in a single run. Obtaining high yields with short synthesis times is even more critical for molecular imaging biomarkers bearing positron-emitting radioisotopes with shorter half-lives, such as ^{11}C ($t_{1/2}$ = 20 min) and ^{13}N ($t_{1/2}$ = 10 min). A unique aspect of PET molecular imaging probes is that only nanogram masses per dose of the radiopharmaceuticals are administered to subjects.

The radiopharmaceutical requirements of expedited chemical kinetics and low-mass quantities of product, together with the emerging need to expand and diversify the catalog of molecular imaging probes, provide a unique opportunity for the use of integrated microfluidics. In addition, the preparation of [^{18}F]FDG provides a conceptual model for the preparation of other molecules (including pharmaceuticals) because it includes common steps required in many chemical syntheses.

We developed a microfluidic chemical reaction circuit (Fig. 1) capable of executing the five chemical processes of the syntheses of both [^{18}F]FDG and [^{19}F]FDG within a nanoliter-scale reaction vessel. Conceptually, however, the chip was designed to demonstrate the digital control of sequential chemical steps, variable chemical environments, and variable physical conditions, all on a single chip. It was also designed to produce sufficient quantities of [^{18}F]FDG (100 to 200 μCi) for mouse imaging. The chip thus has the capability of synthesizing the equivalent of a single mouse dose of [^{18}F]FDG on demand. The device accelerates the synthetic process and reduces the quantity of reagents and solvents required. This integrated microfluidic chip platform can be extended to other radiolabeled molecular imaging probes.

Some of the components required for conducting sequential chemical processes within microfluidics are similar to those previously demonstrated for biological analysis: isolation of distinct regions on the chip with micromechanical valves for nanoliter chemical reactions (*15*), acceleration of diffusion-dominated mixing within a confined volume with a rotary pump (*16*), and creation of in situ affinity columns (*10*). However, two additional technical advances were required to perform effective chemical synthesis. First, an in situ ion exchange column was combined with a rotary pump to concentrate radioisotopes by nearly three orders of magnitude, thereby optimizing the kinetics of the desired reactions. Second, the gas-permeable poly(dimethylsiloxane) (PDMS) matrix allows solvent exchange to occur within the microfluidic channel through direct evaporation, thereby allowing for the sequential execution of chemical reactions in PDMS-compatible solvents (*17*). A solution inside a PDMS-based microfluidic reactor can be heated above its normal (atmospheric) boiling point to provide further kinetic enhancement. Pressure is mediated not only by the heat supplied to the chip, but also by the porosity of the PDMS matrix. Thus, PDMS plays a role akin to the safety valve of a pressure cooker that regulates the "cooking pressure" within a critical range. Our device permits computer-controlled mixing of spatially isolated reagents under individually regulated solvent and temperature conditions.

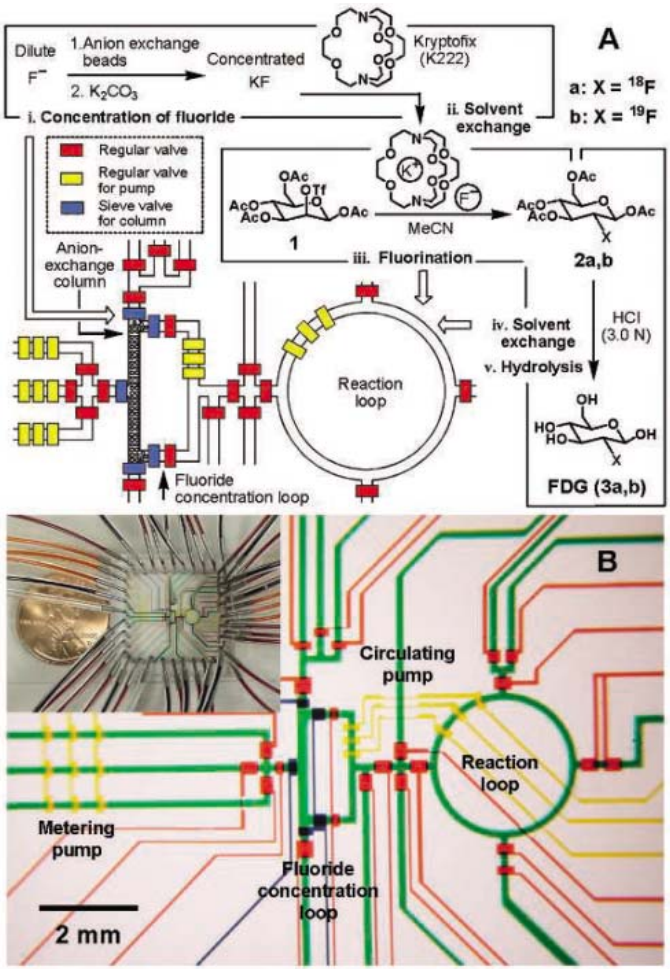

Fig. 1. (**A**) Schematic representation of a chemical reaction circuit used in the production of 2-deoxy-2-fluoro-D-glucose (FDG). Five sequential processes are shown: (i) concentration of dilute fluoride ion with the use of a miniaturized anion exchange column located in a rectangle-shaped fluoride concentration loop, (ii) solvent exchange from water to dry MeCN, (iii) fluorination of the D-mannose triflate precursor **1**, (iv) solvent exchange back to water, and (v) acidic hydrolysis of the fluorinated intermediate **2a** (or **2b**) in a ring-shaped reaction loop. Nanogram amounts of FDG (**3a**, **3b**) are the final product. The operation of the circuit is controlled by pressure-driven valves, with their delegated responsibilities illustrated by their colors: red for regular valves (for isolation), yellow for pump valves (for fluidic metering circulation), and blue for sieve valves (for trapping anion exchange beads in the column module). (**B**) Optical micrograph of the central area of the circuit. The various channels have been loaded with food dyes to help visualize the different components of the microfluidic chip; colors are as in (A), plus green for fluidic channels. Inset: Actual view of the device; a penny (diameter 18.9 mm) is shown for comparison.

The production (*12*) of [^{18}F]FDG is based on five sequential chemical processes (Fig. 1A): (i) concentration of the dilute [^{18}F]fluoride mixture (*18*) solution (<1 ppm, specific activity ~5000 to 10,000 Ci/mmol), obtained from the proton bombardment of [^{18}O]water at a cyclotron facility (*19*); (ii) solvent exchange from water to acetonitrile (MeCN); (iii) [^{18}F]fluoride substitution of the triflate group in the D-mannose triflate precursor **1** in dry MeCN; (iv) solvent exchange from MeCN to water; and (v) acidic hydrolysis of the fluorinated intermediate **2a** to obtain [^{18}F]FDG (**3a**).

The concentration of [^{18}F]fluoride mixture (*18*) obtained from a proton bombardment of [^{18}O]water is usually below 1 ppm. We created a miniaturized anion exchange column (Fig. 2) in the microfluidic device to concentrate the [^{18}F]fluoride mixture solution to ~100 ppm. Sieve valves (Fig. 2B) were created using a square-profiled fluidic channel and a control membrane. Actuation of this membrane prohibits the passage of large particles while still permitting the solution to pass along the edges of the channel. Using these sieve valves to trap anion exchange beads, we obtained the anion exchange column (Fig. 2, C and D) for the concentration of the [^{18}F]fluoride mixture.

We performed a proof-of-concept trial (Fig. 3) with the use of nonradioactive [^{19}F]fluoride. The acquired experimental parameters could be used directly for the production of radioactive [^{18}F]FDG. For the concentration of dilute fluoride (process i, Fig. 1A), a NaF solution (5 ppm) was loaded into the anion exchange column (Fig. 3A). The loading rate (5.0 nl/s) was controlled with a metering pump. After the fluoride solution was loaded completely, a K_2CO_3 solution (0.25 M, 18 nl) was introduced to fill the rectangular loop. The circulating pump module was then turned on so that the K_2CO_3 solution (0.25 M, 18 nl) could loop through the column continuously to produce a concentrated KF solution.

Because the fluorination (process iii) of the D-mannose triflate precursor requires anhydrous conditions, a digitally controlled hot plate was used to heat the reaction circuit for removing water (process ii) from the concentrated KF

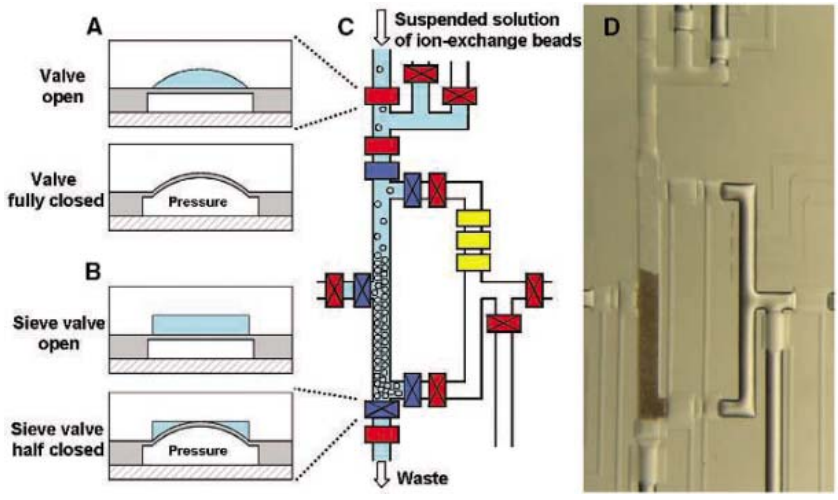

Fig. 2. Schematic representations of the operational mechanisms of (**A**) a regular valve having a round-profiled fluidic channel, and (**B**) a sieve valve having a square-profiled fluidic channel. When pressure is introduced into the control channels, the elastic membranes expand into the fluidic channels. In a regular valve, the fluidic channel is completely sealed because of the perfect fit between the expanded membranes and the round profile of the fluidic channel. In a sieve valve, the square-profiled fluidic channel is only partially closed, which allows fluid to flow along the two edges. Sieve valves can be used to confine solid objects within the fluidic channel but allow liquid to flow through. (**C**) Schematic illustration of the loading of anion exchange beads into a column module incorporating one fluidic channel and five sieve and five regular valves (×, closed valve). A suspended solution of anion exchange beads is introduced into the column modules where five sieve valves and five regular valves operate cooperatively to trap anion exchange beads inside the fluidic channel (total volume 10 nl). A miniaturized anion exchange column for fluoride concentration is achieved when the fluidic channel is fully loaded. (**D**) A snapshot of the bead-loading process in action.

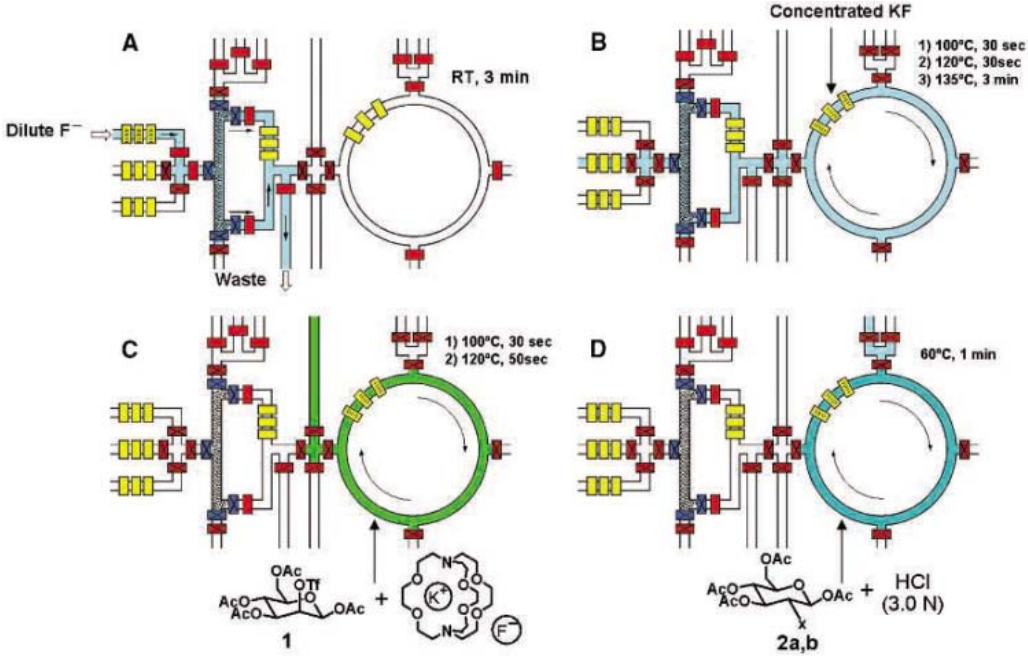

Fig. 3. Schematic diagrams showing the four most critical steps of FDG production in the reaction circuit. (**A**) Concentration of dilute fluoride ion. With the cooperation of regular valves, a dilute fluoride solution (blue) is introduced into the ion exchange column by a metering pump. (**B**) Evaporation of water from the concentrated KF solution. After transferring the concentrated KF solution from the fluoride concentration loop to the ring-shaped reaction loop, the circuit is heated on a hot plate to evaporate water from the reaction loop. Meanwhile, all of the surrounding regular valves are completely closed and the circulating pump is turned on. (**C**) Fluorination reaction. After introduction of a MeCN solution (green) of Kryptofix and the D-mannose triflate **1** into the reaction loop, the inhomogeneous reaction mixture is isolated in the reaction loop, mixed using the circulating pump, and heated under a computer-controlled gradient to generate the intermediate **2a** (or **2b**). (**D**) Hydrolysis reaction. After evaporation of the MeCN, an HCl solution (blue) is introduced into the reaction loop to hydrolyze the intermediate **2a** (or **2b**) to give the final product, FDG (**3a**, **3b**).

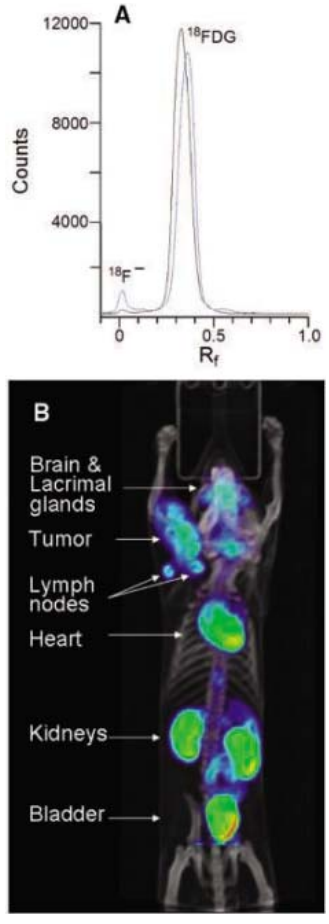

Fig. 4. (**A**) Analytical TLC profile of the unpurified mixture (blue curve) obtained upon the production of [^{18}F]FDG in the second-generation reaction circuit, indicating that the radiochemical purity of the FDG production is 96.2%. The two peaks have R_f values of 0.0 and 0.36, corresponding to [^{18}F]fluoride and [^{18}F]FDG, respectively. After purification and sterilization, the [^{18}F]FDG (black curve) with 99.3% radiochemical purity was used for mouse microPET/microCT imaging. (**B**) Projection view of microPET/microCT image of a tumor-bearing mouse injected with [^{18}F]FDG produced in a microfluidic chip.

solution (Fig. 3B). Dry MeCN was loaded into the reaction loop and the reaction circuit was heated again to completely extrude any remaining moisture. Moisture and MeCN vapor can penetrate and escape the gas-permeable PDMS matrix. Once the circuit had cooled to room temperature, an anhydrous MeCN solution (40 nl) containing the D-mannose triflate **1** (92 ng, limiting reagent) and Kryptofix 222 (364 ng) was introduced into the ring-shaped reaction loop containing the dried KF. This heterogeneous reaction mixture was mixed inside the loop using the circulating pump. During this step (process iii), the circuit was heated (100°C for 30 s and then 120°C for 50 s) to yield the fluorinated intermediate **2b** (Fig. 3C), as analyzed by gas chromatography–mass spectrometry (GC-MS). This analysis indicated that the conversion yield for the fluorination process was 98%. After removal of MeCN by direct evaporation, 3 N HCl solution (40 nl) was injected into the circuit, and the hydrolysis (Fig. 3D, processes iv and v) of the intermediate **2b** was conducted at 60°C to obtain [^{19}F]FDG in >90% purity, according to GC-MS analysis. The entire synthesis was reproduced on multiple chips.

Radioactive [^{18}F]FDG was also produced in the reaction circuit by starting from the radioactive [^{18}F]fluoride mixture. For this demonstration, only 720 μCi of [^{18}F]fluoride (limiting reagent) in ~1 μl of [^{18}O]water was used. Because of the relatively high loading rate (~65 nl/s) applied, only 500 μCi of [^{18}F]fluoride was trapped in the column. Then, 324 ng of D-mannose triflate was introduced into the circuit to obtain 190 μCi of [^{18}F]FDG with a radiochemical yield of 38% and a radiochemical purity of 97.6% (*20*), according to radio–thin layer chromatography (TLC) analysis. This sequential production of [^{18}F]FDG was completed in automated fashion within 14 min (*21*), and similar results were observed across multiple runs.

We also designed a second-generation chemical reaction circuit with the capacity to synthesize larger [^{18}F]FDG doses (*22*). This chip has a coin-shaped reactor (volume 5 μl) equipped with a vacuum vent. It was used to synthesize 1.74 mCi of [^{18}F]FDG, an amount sufficient for several mouse experiments. From the purified and sterilized product (Fig. 4A), two doses (375 μCi and 272 μCi) were used for microPET- and microCT-based molecular imaging of two mouse models of cancer (*23*). One of the mouse images is shown in Fig. 4B as a two-dimensional projection. This circuit design should ultimately yield large enough quantities (i.e., >100 mCi) of [^{18}F]FDG for multiple human PET scans, which typically use 10 mCi per patient.

A major limitation (*17*) of the current chips involves the PDMS elastomer. This material is not chemically resistant to most organic solvents, so it seriously limits the variety of chemical reactions that can be executed within integrated microfluidic environments. New solvent-resistant elastomeric materials (*24*) have been introduced for integrated microfluidic chips. Therefore, the above technology shows promise for a broad range of chemical syntheses. In addition to the flexibility of rapidly arranging unit operations on an integrated microfluidic chip for specific reactions, new circuit designs take less than 2 days to proceed from a computer-aided design (CAD)–based proposal to a working chip. Taken together with the low production costs for the chips, these chemical reaction circuits offer an appealing versatility for molecular biomarker and pharmaceutical chemistry, among other applications.

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17. Solvent incompatibility of PDMS limits the application of PDMS-based integrated microfluidics for performing organic reactions. When PDMS-based devices are exposed to certain organic solvents (e.g., hydrocarbons, toluene, and chloroform), the PDMS materials swell, leading to delamination and malfunction of the devices. Nonetheless, some low-solubility organic solvents (e.g., acetonitrile, dimethylsulfoxide, and glycerol) are compatible with performing organic reactions in microfluidic channels made in PDMS. For a study on solvent compatibility of PDMS, see (*25*).
18. [^{19}F]Fluoride ion (~0.2 ppm) is an ubiquitous contaminant in [^{18}O]water, and thus [^{18}F]fluoride ion produced from it will have a total fluoride (radioactive + nonradioactive) ion concentration of about ~0.2 ppm. The concentration of [^{18}F] fluoride ion in this mixture is generally <10 parts per thousand.
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20. Radioactive reaction wastes, including unreacted [^{18}F]fluoride and contaminated microfluidic chips, were stored in a lead-shielded area until they decayed to background levels, which takes about 24 hours.
21. The complete 14-min synthesis of FDG inside the circuit was recorded and compressed into a 2-min movie clip (movie S1).
22. See supporting data on *Science* Online.
23. The tumor model used was a strongly immunogenic, nonmetastasizing, retrovirally induced rhabdomyosarcoma (M-MSV/M-MuLV). MSV is a replication-defective, acutely transforming retrovirus carried with helper activity provided by M-MuLV, which encodes the gag, pol, and env components that are necessary for cell infection and replication. Rhabdomyosarcomas develop at the intramuscular inoculation site after a short latency period (7 to 10 days) and regress over a period of 4 to 5 weeks after the induction of a strong immune reaction in immunocompetent adult mice.
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26. Supported by the U.S. Department of Energy–funded Institute for Molecular Medicine laboratory, the National Cancer Institute, the Norton Simon Research Foundation, the UCLA SOMI training program, NIH training grant 5T32-GM07616, and a collaboration between Molecular Imaging/Siemens and Fluidigm. We thank H. Padgett at Siemens for his contribution in radiochemical experiments.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5755/1793/DC1
Materials and Methods
Figs. S1 to S7
Movie S1
References

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Direct Experimental Evidence of a Growing Length Scale Accompanying the Glass Transition

L. Berthier,[1]* G. Biroli,[2] J.-P. Bouchaud,[3,4] L. Cipelletti,[1] D. El Masri,[1] D. L'Hôte,[4] F. Ladieu,[4] M. Pierno[1]

Understanding glass formation is a challenge, because the existence of a true glass state, distinct from liquid and solid, remains elusive: Glasses are liquids that have become too viscous to flow. An old idea, as yet unproven experimentally, is that the dynamics becomes sluggish as the glass transition approaches, because increasingly larger regions of the material have to move simultaneously to allow flow. We introduce new multipoint dynamical susceptibilities to estimate quantitatively the size of these regions and provide direct experimental evidence that the glass formation of molecular liquids and colloidal suspensions is accompanied by growing dynamic correlation length scales.

Why does the viscosity of glass-forming liquids increase so dramatically when approaching the glass transition? Despite decades of research, a clear explanation of this phenomenon, common to materials as diverse as molecular glasses, polymers, and colloids, is still lacking (*1*, *2*). The conundrum is that the static structure of a glass is indistinguishable from that of the corresponding liquid, with no sign of increasing static correlation length scales accompanying the glass transition. Numerical simulations performed well above the glass temperature, T_g, reveal instead the existence of a growing dynamic length scale (*3*–*7*) associated with dynamic heterogeneities (*8*). Experiments (*8*–*12*) have indirectly suggested a characteristic length scale of about 5 to 20 molecular diameters at T_g, but its time and temperature dependencies, which are crucial for relating this finding to the glass transition, were not determined.

We present quantitative experimental evidence that glass formation in molecular liquids and colloids is accompanied by at least one growing dynamic length scale. We introduce experimentally accessible multipoint dynamic susceptibilities that quantify the correlated nature of the dynamics in glass formers. Because these measurements can be made using a wide variety of techniques in vastly different materials, a detailed characterization of the microscopic mechanisms governing the formation of amorphous glassy states becomes possible.

Supercooled liquids are believed to exhibit spatially heterogeneous dynamics over length scales that grow when approaching the glass state (*1*, *13*–*15*). This heterogeneity implies the existence of significant fluctuations of the dynamics, because the number of independently relaxing regions is reduced. Numerical simulations have focused on a "four-point" dynamic susceptibility $\chi_4(t)$, which quantifies the amplitude of spontaneous fluctuations around the average dynamics (*3*–*7*). The latter is usually measured through ensemble-averaged correlators, $F(t) = \langle \delta A(t) \delta A(0) \rangle = \langle C(t) \rangle$, where $\delta A(t) = A(t) - \langle A \rangle$ represents the spontaneous fluctuation of an observable $A(t)$, such as the density. Dynamic correlation leads to large fluctuations of $C(t)$, measured by $\chi_4(t) = N\langle \delta C^2(t) \rangle$, where N is the number of particles in the system. The susceptibility $\chi_4(t)$ typically presents a nonmonotonic time dependence with a peak centered at the liquid's relaxation time (*16*). The height of this peak is proportional to the volume within which correlated motion takes place (*4*, *5*, *15*, *16*). Unfortunately, numerical findings are limited to short time scales ($\sim 10^{-7}$ s) and temperatures far above T_g. Experimentally, detecting spontaneous fluctuations of dynamic correlators remains an open challenge, because dynamic measurements have to be resolved in both space and time (*17*).

Induced fluctuations are more easily accessible experimentally than spontaneous ones and can be related to one another by fluctuation-dissipation theorems. We introduce a dynamic susceptibility defined as the response of the correlator $F(t)$ to a perturbing field x.

$$\chi_x(t) = \frac{\partial F(t)}{\partial x} \qquad (1)$$

The relaxation time of supercooled liquids increases abruptly upon cooling, so a relevant perturbing field is temperature, in which case Eq. 1 becomes $\chi_T(t) = \partial F(t)/\partial T$. Density also plays a role in supercooled liquids, although a less crucial one (*18*). Hence, another interesting susceptibility is $\chi_P(t) = \partial F(t)/\partial P$, where P is the pressure. Colloidal hard spheres undergo a glass transition (*19*) at high particle volume fraction φ. Thus, the appropriate susceptibility for colloids is $\chi_\varphi(t) = \partial F(t)/\partial \varphi$. Equation 1 also applies in the frequency domain, $\chi_x(\omega) = \partial \tilde{F}(\omega)/\partial x$, where $\tilde{F}(\omega)$ can be the dielectric susceptibility. We will show below that linear response formalism and fluctuation theory can be used to relate $\chi_x(t)$ to the spontaneous fluctuations of $C(t)$, and thus to $\chi_4(t)$. Thus, $\chi_x(t)$ is an experimentally accessible multipoint dynamic susceptibility that directly quantifies dynamic heterogeneity in glass formers.

For molecular liquids, the dynamics conserves energy, volume, and number N of particles, and one can establish, in the *NPT* ensemble relevant for experiments, the following fluctuation-dissipation theorem

$$k_B T^2 \chi_T(t) = N\langle \delta C(t) \delta H(0) \rangle \qquad (2)$$

where k_B is the Boltzmann constant, $H(t)$ the fluctuating enthalpy per particle, and $C(t)$ the instantaneous value of a generic dynamic correlator $F(t)$. Both $C(t)$ and $H(t)$ are sums over local contributions (*20*), $NC(t) = \rho \int d^3\boldsymbol{r} c(\boldsymbol{r},t)$ and $NH(t) = \rho\sqrt{k_B c_P} T \int d^3\boldsymbol{r} \hat{\boldsymbol{h}}(\boldsymbol{r},t)$. Here, ρ is the average number density, c_P the constant pressure specific heat that sets the scale of the enthalpy fluctuations, $\langle \delta H^2 \rangle = k_B c_P T^2$, so that the field $\hat{h}(\boldsymbol{r},t)$ has unit variance. Using translational invariance, Eq. 2 can be rewritten as:

$$\sqrt{\frac{k_B}{c_P}} T \chi_T(t) = \rho \int d^3\boldsymbol{r} \left\langle \delta c(\boldsymbol{r},t) \delta \hat{h}(\boldsymbol{0},0) \right\rangle \qquad (3)$$

This expression shows that $\chi_T(t)$ directly probes the range of spatial correlations between local fluctuations of the dynamics and that of the enthalpy. In the case of colloids, the dynamics only conserves density, and a similar expression can be obtained

$$\sqrt{\rho k_B T \kappa_T}\, \varphi \chi_\varphi(t) = \rho \int d^3\boldsymbol{r} \left\langle \delta c(\boldsymbol{r},t) \delta \hat{\rho}(\boldsymbol{0},0) \right\rangle \qquad (4)$$

where κ_T is the isothermal compressibility and $\delta\hat{\rho}$ denotes density fluctuations rescaled by their root mean square.

Equations 3 and 4 show that $\chi_x(t)$ probes the extent of spatial dynamic correlations that differ from the ones studied in earlier theoretical and numerical works, which focused instead on

[1]Laboratoire des Colloïdes, Verres, et Nanomatériaux, UMR 5587, Université Montpellier II and CNRS, 34095 Montpellier, France. [2]Service de Physique Théorique Orme des Merisiers, CEA Saclay, 91191 Gif sur Yvette Cedex, France. [3]Science and Finance, Capital Fund Management 6-8 Bd Haussmann, 75009 Paris, France. [4]Service de Physique de l'État Condensé Orme des Merisiers, CEA Saclay, 91191 Gif sur Yvette Cedex, France.

*To whom correspondence should be addressed. E-mail: berthier@lcvn.univ-montp2.fr

$\chi_4(t) = \rho\int d^3\mathbf{r}\langle\delta c(\mathbf{r},t)\,\delta c(\mathbf{0},t)\rangle$. We have, however, established a direct relation between $\chi_x(t)$ and $\chi_4(t)$ by using the thermodynamic formalism developed in (*21*), which is generically applicable to bulk glass formers above the glass transition. For dynamics conserving energy and volume, we relate the fluctuations of $C(t)$, and therefore $\chi_4(t)$ measured in the *NPT* ensemble, to its isobaric-isoenthalpic counterpart, $\chi_4^{NPH}(t)$, which quantifies the amplitude of the fluctuations of $C(t)$ in the *NPH* ensemble in which all configurations have exactly the same enthalpy: $\chi_4(t) = \chi_4^{NPH}(t) + k_BT^2\chi_T^2(t)/c_P$. Because $\chi_4^{NPH}(t) > 0$, one derives an experimentally measurable rigorous lower bound (*22*) for $\chi_4(t)$.

$$\chi_4(t) \geq \frac{k_B}{c_P}T^2\chi_T^2(t) \qquad (5)$$

A similar inequality holds between $\chi_4(\omega)$ and $\chi_T(\omega)$, where $\chi_4(\omega)$ denotes the amplitude of spontaneous fluctuations around $\tilde{F}(\omega)$. Similar arguments also apply to $\chi_4(t)$, computed in the *NVT* ensemble preferred in numerical simulations. In that case, energy replaces enthalpy in Eqs. 2 and 3, and the specific heat at constant volume, c_V, replaces c_P in Eq. 3 and relation 5. Finally, we find that an inequality similar to relation 5 holds for colloidal systems, for which the volume and the number of particles are conserved quantities.

$$\chi_4(t) \geq \rho k_BT\kappa_T\varphi^2\chi_\varphi^2(t) \qquad (6)$$

We have determined $\chi_x(t)$ experimentally and numerically in three representative glass formers. For supercooled glycerol near $T_g \approx 185$ K, the real part of the dielectric susceptibility, $\varepsilon'(\omega)$, was measured every 1 K in the temperature range from 192 to 232 K. After fitting to a Havriliak-Negami form (*1*), we use smoothed finite differences to evaluate $\chi_T(\omega) = \partial[\varepsilon'(\omega)/\varepsilon'(0)]/\partial T$ and show in Fig. 1A the right side of relation 5 as a function of inverse frequency. We plot in Fig. 1B the right side of relation 6 for hard sphere colloids where $\chi_\varphi(t) = \partial f(q,t)/\partial\varphi$. The normalized intermediate scattering function (*20*) $f(q,t)$ is measured by dynamic light scattering (*23*) for a wave vector q close to the first peak of the static structure factor. Several packing fractions are studied, from diluted samples where $f(q,t)$ decays exponentially in ~1 ms to concentrated suspensions with a two-step decay and a final relaxation time of ~10 s. Finite differences of data sets obtained for nearby φ are used to deduce $\chi_\varphi(t)$. Finally, we show in Fig. 1C numerical data obtained by standard molecular dynamics simulations of a binary Lennard-Jones mixture, a well-studied model for fragile supercooled liquids (*24*, *25*). The dynamics is recorded at nearby temperatures through the self part of the intermediate scattering function, whose characteristic decay time spans a range from 1 ps to 100 ns [using Argon units (*24*, *25*)].

Dynamical susceptibilities behave similarly in all three cases. All display a peak for $t \approx \tau_\alpha$, the average relaxation time. The peak height increases when the glass transition is approached. This behavior represents the central result of our work. Together with Eqs. 3 to 6, it provides direct evidence of enhanced dynamic fluctuations and a growing dynamic length scale associated with the glass transition.

How tight the bounds of relations 5 and 6 are depends on the specific material and range of parameters studied. A quantitative answer is given by simulations where the microcanonical quantity $\chi_4^{\mathrm{micro}}(t)$, i.e., the difference between $\chi_4(t)$ in the *NVT* ensemble and $k_BT^2\chi_T(t)/c_V$, can be easily measured. For the Lennard-Jones mixture, we find that the right side of relation 5 is much smaller than $\chi_4(t)$ at high T, but the difference rapidly diminishes when T decreases. Both sides of relation 5 become comparable for the lowest temperature shown in Fig. 1C, which is still well above T_g. Following (*26*), we also find that mode-coupling theory predicts $\chi_4(t) \sim \chi_T^2(t) \sim (T/T_c - 1)^{-2}$ near the mode-coupling singularity $T_c > T_g$, provided that conserved variables are properly taken into account.

These results support the idea that relation 5 can be used as an equality to quantitatively estimate $\chi_4(t)$ at low temperature, at least for fragile systems. This use of relation 5 is equivalent to assuming that dynamic heterogeneity in molecular liquids is strongly correlated with enthalpy fluctuations and, through a similar argument, with density fluctuations in colloids. In fact, supposing that enthalpy is the only source of fluctuations, $\delta C \approx (\partial C/\partial T)_P\,\delta H/c_P$, and if we use the definition of c_P, we obtain directly that $\chi_4(t) \approx k_BT^2\chi_T^2(t)/c_P$. A more general result can be obtained by taking into account that energy and density are both fluctuating quantities, in which case $\chi_4(t)$ is the sum of two contributions: $\chi_4(t) \approx k_BT^2\chi_T^2(t)/c_V + \rho k_BT\kappa_T\rho^2\chi_\rho^2(t)$. The second term is negligible in most fragile liquids (*18*) but dominates in colloidal systems. The presence of addition-

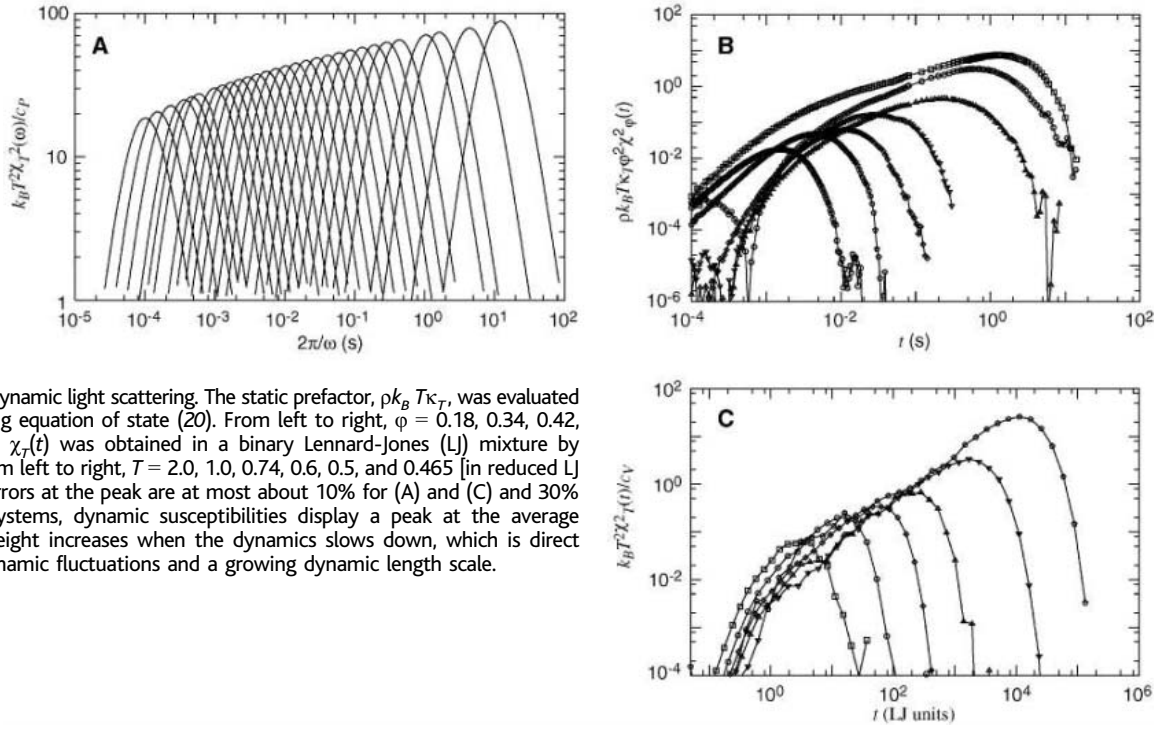

Fig. 1. Dynamic susceptibilities in "χ_4 units," right side of relations 5 and 6 for three glass formers. (**A**) $\chi_T(\omega)$ was obtained for 99.6% pure supercooled glycerol in a desiccated Argon environment to prevent water absorption by using standard capacitive dielectric measurements for 192 K $\leq T \leq$ 232 K ($T_g \approx 185$ K). (**B**) $\chi_\varphi(t)$ was obtained in colloidal hard spheres by dynamic light scattering. The static prefactor, $\rho k_B T\kappa_T$, was evaluated from the Carnahan-Starling equation of state (*20*). From left to right, φ = 0.18, 0.34, 0.42, 0.46, 0.49, and 0.50. (**C**) $\chi_T(t)$ was obtained in a binary Lennard-Jones (LJ) mixture by numerical simulation. From left to right, T = 2.0, 1.0, 0.74, 0.6, 0.5, and 0.465 [in reduced LJ units (*24*, *25*)]. Relative errors at the peak are at most about 10% for (A) and (C) and 30% for (B). For all of the systems, dynamic susceptibilities display a peak at the average relaxation time whose height increases when the dynamics slows down, which is direct evidence of enhanced dynamic fluctuations and a growing dynamic length scale.

al sources of fluctuations justifies that the rigorously derived inequality 5 does not hold as an equality.

Our results for dynamic fluctuations provide an estimate of the size ξ of dynamic heterogeneity in liquids near T_g. Because $\chi_4(t) = \rho\int d^3\boldsymbol{r}\ \langle \delta c(\boldsymbol{r},t)\delta c(\boldsymbol{0},t)\rangle$, this quantity, once divided by the amplitude of the fluctuations at zero distance, $\langle \delta c^2(\boldsymbol{0},t)\rangle$, defines a correlation volume. The correlation functions are normalized to unity at $t = 0$, so $\langle \delta c^2(\boldsymbol{0},t)\rangle$ is on the order of 1 or smaller. Our simulations indeed show that in the temperature regime where the dynamics slows down and on time scales not much longer than the system relaxation time, this average is on the order of 1 and displays extremely weak temperature dependence, as expected for a local quantity in glass formers. Thus, the height of the peak in the dynamic susceptibility, $\chi_4^* \approx k_B\ T^2(\chi_T^*)^2/c_P$, yields directly a correlation volume expressed in molecular units, $\chi_4^* \cong (\xi/a)^\zeta$, where a is the molecular size and c_P is expressed in units of k_B. Numerical (*4*, *6*) and theoretical (*6*, *15*, *16*, *26*) works suggest that $\zeta \approx 2$ to 4.

A direct comparison between our data and existing measurements can be performed for glycerol, where multidimensional nuclear magnetic resonance (NMR) experiments show that $\xi = 1.3 \pm 0.5$ nm for $T = 199$ K (*27*, *28*). Assuming a simple compact geometry for heterogeneities, $\zeta = 3$, we estimate that ξ increases from 0.9 nm at $T = 232$ K to 1.5 nm at 192 K. Given the assumptions involved in both approaches, and the uncertainty about numerical prefactors of order unity, the agreement is remarkable. An important physical conclusion of our work is that dynamic heterogeneity is strongly correlated to enthalpy fluctuations in fragile liquids, although there is no signature of any static large-scale correlations (*3*, *6*, *15*).

For other glass-forming liquids, we obtain an estimate for ξ at T_g by assuming for simplicity that correlators obey time-temperature superposition, $F(t) = \mathcal{F}(t/\tau_\alpha)$, and using relation 5 as an equality. One gets

$$\chi_4^*(T_g) \approx [\mathcal{F}'(1)]^2 \frac{k_B}{c_p}\left(\frac{\partial \ln \tau_\alpha}{\partial \ln T}\Big|_{T_g}\right)^2 \quad (7)$$

The logarithmic derivative is proportional to the well-known "steepness index" m, introduced in the glass literature to characterize the fragility of glass-forming liquids (*29*). From reported values (*1*, *29*) of the quantities appearing in Eq. 7 and assuming a stretched exponential form for $\mathcal{F}(x) = \exp(-x^\beta)$, we estimate $\chi_4^*(T_g)$ for different glass-forming liquids in Fig. 2A.

For complex molecules, fluctuations that are unrelated to the glassy dynamics might contribute to the specific heat. These effects may be taken into account by replacing c_P in Eq. 7 by Δc_P, the jump in specific heat at T_g, which is sensitive only to the glassy degrees of freedom. Furthermore, for large molecules, the molecular size is probably not the relevant microscopic length scale, and it is sensible to express the specific heat in units of k_B per "independent bead" instead of molecular units (*30*). These physical assumptions are used in Fig. 2B, where we have converted our results into length scales expressed in bead units, and they lead to a trend similar to that of the main plot but with less scatter: Dynamic correlations revealed by χ_T increase weakly with fragility (*31*). This result is compatible with some theoretical approaches (*32*) but contrasts with others that predict an opposite trend (*33*). This discrepancy might arise from the existence of at least two physically distinct dynamic length scales, one revealed by χ_T and a second associated to χ_4. Although we found that both quantities are comparable for fragile systems, the bound in relation 5 may underestimate χ_4 for strong materials.

To further test our length scale estimate of Eq. 7, we apply the formula to a polymeric liquid poly(vinyl acetate) (PVAc) using the monomer size for a (*27*). We find $\xi \approx 2.0$ nm at T_g to be compared with the value of 3.7 ± 1 nm obtained at $T_g + 10K$ (*9*, *28*) [we assume $\zeta = 3$ and use the data on PVAc in (*9*, *28*)]. Again, the agreement is satisfactory. A similar agreement is found for orthoterphenyl and sorbitol, for which available NMR data are reported in Fig. 2A. Hence, we find that typical values for the dynamic correlation length at T_g obtained by Eq. 7 are in good agreement with previous experiments performed near T_g (*1*, *8*, *9*, *26*, *34*). However, our approach has a broader scope, because it allows one to extend experimental studies of dynamic heterogeneity to a range of temperatures not previously accessible and to the full-time dependence of the fluctuations (Fig. 1). Finally, we remark that even for (strong) Arrhenius molecular liquids with activation energy E, relation 5 and time temperature superposition give $\chi_4^*(T) \geq (k_B/c_P) \times E^2/(k_B\ T)^2$, showing that dynamic heterogeneity must also exist in that case (*35*), in agreement with the general argument that for systems with finite range interactions, diverging time scales must be accompanied by diverging length scales.

Our experiments provide a quantitative demonstration that dynamic correlations and length scales increase as the glass transition is approached. More work is needed to characterize the time and temperature dependencies of dynamic fluctuations over a larger range of materials and parameters. Open issues also concern the precise space-time geometry of dynamic heterogeneity that fixes the value of the exponent ζ and the relation between time scales and length scales, the connection between cooperativity and heterogeneity, and the extension of our results to the nonequilibrium aging dynamics encountered in the glass phase.

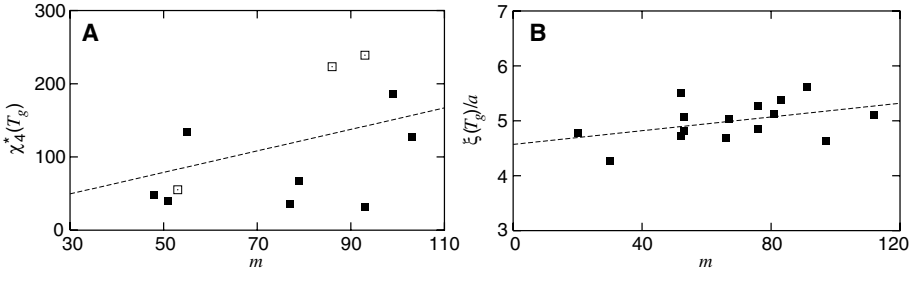

Fig. 2. (**A**) Correlation volume $\chi_4^*(T_g)$ in supercooled liquids at the glass transition. Filled squares represent a lower bound to $\chi_4^*(T_g)$ in molecular units estimated through Eq. 7. Different points represent different materials, which are ranked by their fragility m. Open squares represent the same quantity evaluated from available multidimensional NMR data, using (*28*) $\chi_4^* = \int d^3\ r\ \exp(-2r/\xi)$, for glycerol ($T_g + 10$ K) (*27*), orthoterphenyl ($T_g + 9$ K) (*28*, *34*), and d-sorbitol ($T_g + 7$ K) (*28*). A linear fit to the weak increase of χ_4^* with fragility is shown as a dashed line. (**B**) Correlation length $\xi(T_g)$ in supercooled liquids at the glass transition expressed in bead units a. The correlation volume is first evaluated using Δc_P instead of c_P in Eq. 7. Following (*30*), Δc_P is expressed in k_B "per bead" units, accounting for different molecular shapes and sizes. Using $\zeta = 3$, the result is finally converted into a length scale expressed in bead units. The known empirical correlations (*29*) between m, β, and Δc_P translate into a weak increase of $\xi(T_g)$ with fragility, which we fit with a linear relation shown as a dashed line.

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Developmental Plasticity in the Life History of a Prosauropod Dinosaur

P. Martin Sander* and Nicole Klein

Long-bone histology indicates that the most common early dinosaur, the prosauropod *Plateosaurus engelhardti* from the Upper Triassic of Central Europe, had variable life histories. Although *Plateosaurus* grew at the fast rates typical for dinosaurs, as indicated by fibrolamellar bone, qualitative (growth stop) and quantitative (growth-mark counts) features of its histology are poorly correlated with body size. Individual life histories of *P. engelhardti* were influenced by environmental factors, as in modern ectothermic reptiles, but not in mammals, birds, or other dinosaurs.

Virtually all dinosaurs studied to date show a primary bone type known as fibrolamellar complex in their long bone wall (*1–3*). This bone type indicates fast growth that must have been sustained by a metabolic rate well above that of modern reptiles, if not as high as that of mammals (*1–4*). Dinosaurs for which such data are available grow along a species-specific growth trajectory with little individual variation in rate of growth and final size (*3*, *5–8*), as in mammals (*9*) and birds (*10*). Here, we show that the most common early dinosaur had a life history in which its growth was affected by environmental factors such as climate and food availability [developmental plasticity (*11*)].

P. engelhardti is found in several mass accumulations of medium to large individuals in the Norian of central Europe, such as Trossingen (southern Germany) and Frick (northern Switzerland) (*12–15*). *Plateosaurus* belongs to a group known as prosauropods, which flourished from the Upper Triassic to the Lower Jurassic, representing the dominant herbivores in faunas of this age worldwide (*15*). Prosauropod interrelationships are controversial (*15–18*), but prosauropods and sauropods together form a monophyletic group, Sauropodomorpha. At a maximum length of 10 m and a corresponding mass of nearly 4 tons, *Plateosaurus* was one of the larger bodied prosauropods. Together with some other prosauropods, this dinosaur was the first to reach the large body size generally attributed to dinosaurs, and the first high browser to evolve.

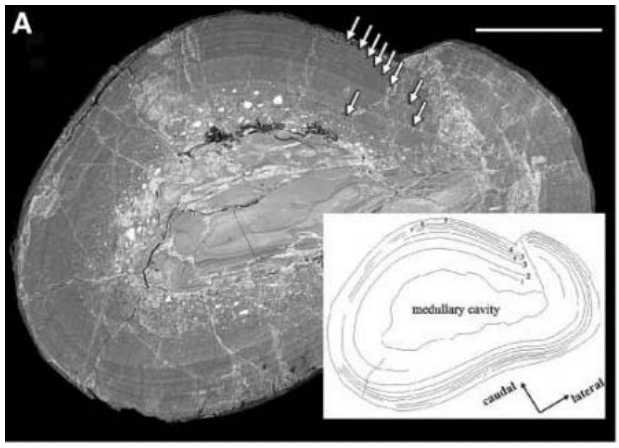
We sampled the histology of long and girdle bones of *P. engelhardti* from Trossingen and Frick (*19*) (table S1). *Plateosaurus* long bones are characterized by a large medullary cavity (49% to 58% of shaft diameter) and relatively thin bone walls (Fig. 1). The cortex is sharply set off from the medullary cavity with little or no secondary cancellous bone and rare resorption spaces in the compact bone. The primary bone of the cortex is dominated by growth cycles of fibrolamellar bone, ending in a line of arrested growth (LAG) (Fig. 2). Vascular canals are primarily circumferential, and vascularity decreases toward the LAG (Figs. 1 and 2). Growth-cycle width decreases substantially toward the outer bone surface (Fig. 1A). In one group of specimens, fibrolamellar bone is the last tissue type to have been formed (Fig. 2). We assigned the ontogenetic stage of "fast growth" to these specimens, because fibrolamellar bone deposition indicates a high growth rate. A strong decrease in growth rate is documented in the last bone tissue deposited in many other specimens, in which growth cycles in fibrolamellar bone in the outer cortex become narrow and less vascularized (Fig. 2). We assigned the "slow growth" stage to this second group. A third group of specimens survived to an even later ontogenetic stage, as evidenced by lamellar-zonal bone with closely spaced LAGs and poor to absent vascularization in the outermost cortex (Fig. 2). This tissue type is also known as an external fundamental system and documents a growth plateau, i.e., that final body size had been reached. Individuals in this group were thus scored as "fully grown."

Surprisingly, we found such fully grown individuals virtually across the whole size range sampled (*19*). Some individuals had reached final size at 4.8 m body length (BL), whereas others attained 10 m BL (Fig. 3). Similarly, the "fast growth" and the "slow growth" stages were also found at widely differing body sizes (Fig. 3). Size at the slow growth stage is close to final body size because not much bone tissue was added to the circumference of the bone during this stage.

Life history was quantified by applying skeletochronology to long and girdle bones (*19*). We estimate that the youngest specimen in the sample was 9 years old, whereas the oldest had reached 26 to 27 years (Fig. 3). This specimen had attained nearly final size but was still growing slowly. The minimum age for a fully grown specimen was 12 years. However, in agreement with our observations about

Institut für Paläontologie, Universität Bonn, Nussallee 8, D-53115 Bonn, Germany.

*To whom correspondence should be addressed. E-mail: martin.sander@uni-bonn.de



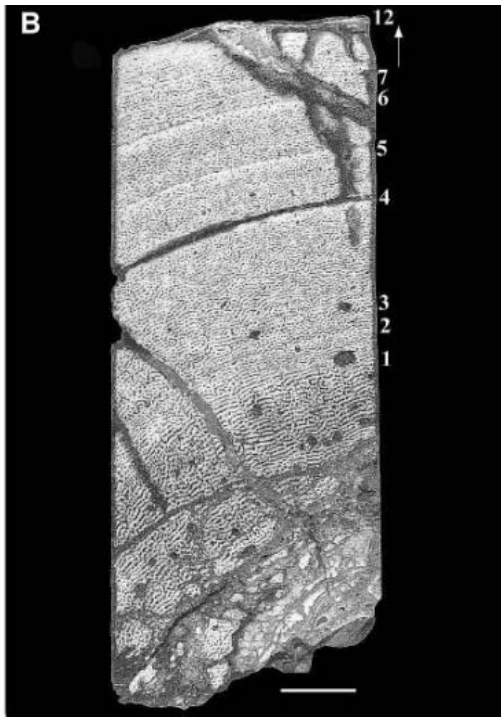


Fig. 1. Histology of *P. engelhardti* long bones, polished sections, normal light. (**A**) Cross section of a humerus shaft (right humerus NAA F 88/B640, 44.5 cm long, Frick). Arrows mark LAGs. Note the decrease in LAG spacing toward the periphery of the bone. Scale bar, 1 cm. (**B**) Core section (left femur IFG compactus, 74 cm long, Trossingen) with growth-mark count. Numbers mark LAGs. IFG, Institut für Geowissenschaften, Universität Tübingen, Germany; NAA, Naturama, Aarau, Switzerland. Scale bar, 3 mm.

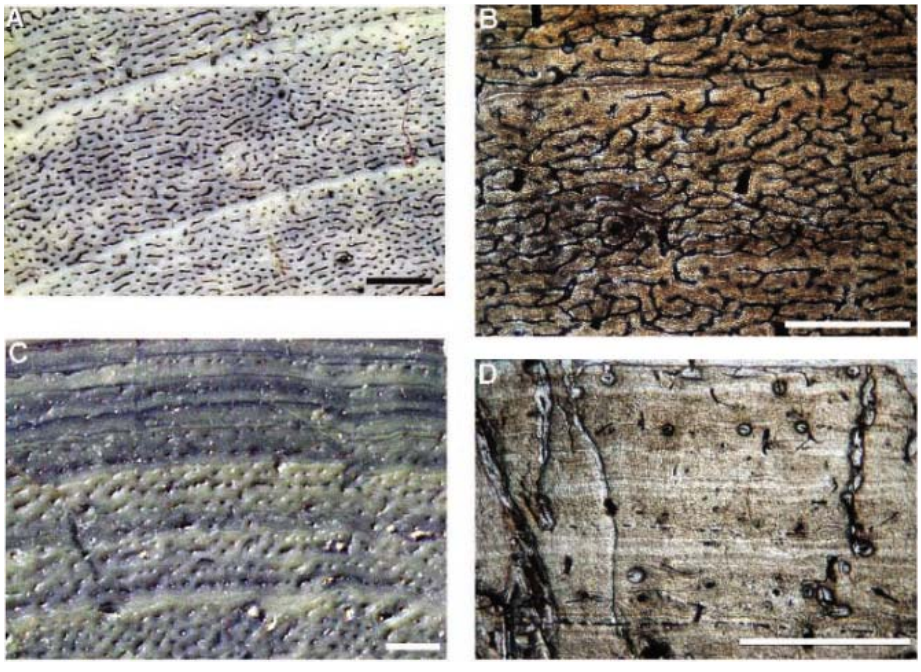


Fig. 2. Ontogenetic stages in the histology of *P. engelhardti* long bones. (**A**) Polished section (left femur IFG compactus, 74 cm long, Trossingen) showing laminar fibrolamellar complex with LAGs. This tissue type indicates fast growth of the cortex. (**B**) Thin section from the same specimen showing the same bone tissue. (**C**) Polished section (right humerus NAA F 88/B640, 44.5 cm long, Frick) showing fibrolamellar bone with LAGs followed by lamellar-zonal bone in the outer cortex. This tissue type indicates slow growth. (**D**) Thin section (left tibia MSF 1, 51 cm long, Frick) with closely spaced LAGs in outermost cortex. This tissue type indicates that the animal was fully grown. Bone surface is beyond [(A) to (C)] or at top (D) of image. IFG, Institut für Geowissenschaften, Universität Tübingen, Germany; MSF, Sauriermuseum, Frick, Switzerland; NAA, Naturama, Aarau, Switzerland. Scale bars, 1 mm.

the qualitative growth record, we found a poor correlation between body size and age (Fig. 3). This is most obvious from the "slow growth stage" sample from Trossingen (n = 19, r = 0.383, no significant correlation at P = 0.001) and the "fully grown" sample from Frick (n = 5, r = 0.114, no significant correlation at P = 0.001).

Two lines of evidence, growth stage assignment and skeletochronology, thus indicate that growth rate and final size varied strikingly in individuals of the species *P. engelhardti*. Hypotheses to explain this extreme variability include: (i) that more than one biological species is represented by the material identified as *P. engelhardti*, (ii) that the material represents a single species with a strong sexual size dimorphism, (iii) that our methods for detecting growth stage and age are inadequate, and (iv) that *P. engelhardti* had strong developmental plasticity in life-history parameters. We view the last interpretation as the most credible. We reject hypothesis (iii), methodological problems, because termination of growth (Fig. 2) can be detected histologically with confidence (*3*, *7*), although our skeletochronological age estimates may be inaccurate because of the lack of juvenile *Plateosaurus* (Fig. 3). We also reject hypothesis (ii), sexual dimorphism, because terminal body size shows a unimodal distribution, albeit with a high standard deviation (Fig. 3), and not the bimodal distribution expected for sexual morphs. Sexual dimorphism in aspects of morphology and in metric characters has been postulated for *Plateosaurus* but cannot be proven (*20*, *21*). Finally, the morphology and systematics of *Plateosaurus* have been intensively studied in recent years, and all authors agree that there is only evidence for one species among the *Plateosaurus* fossils from the rich bone beds of central Europe (*13*–*15*). This leads us to reject hypothesis (i).

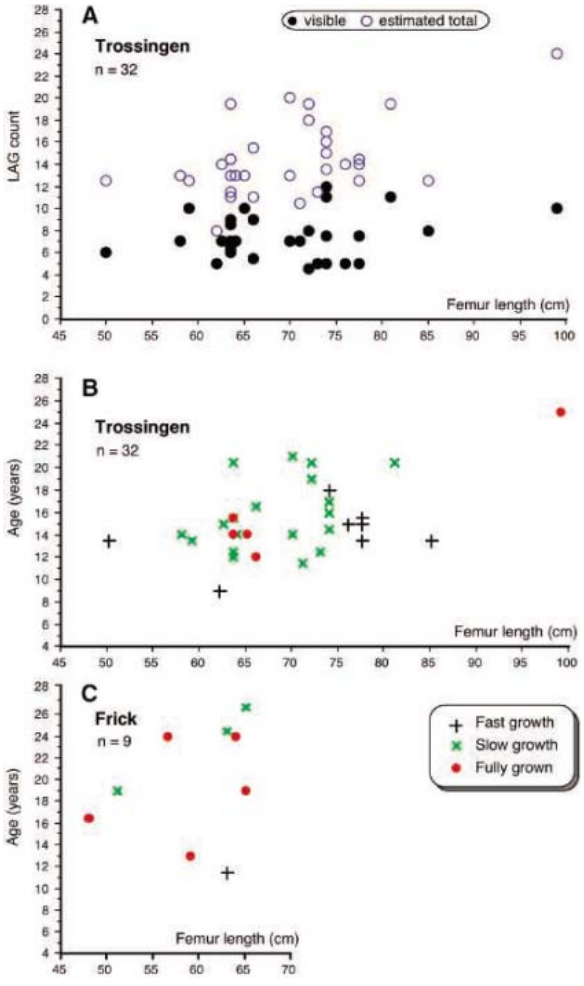


Fig. 3. Relation between body size, age, and ontogenetic stage in specimens of *P. engelhardti*, based on bone histology. Proxy for body size is femur length. Data for plots are in table S1. (**A**) Relation between age and body size in the Trossingen specimens. For each specimen, observed LAG count (black symbols) and estimated total LAG count (blue symbols) are plotted. Estimated total LAG count is equivalent to individual age in years. Note poor correlation between age and size. (**B** and **C**) Relation between growth stage and body size in the samples from Trossingen (B) and Frick (C). Note poor correlation between growth stage and size in both samples. The large variation in final body size is best appreciated if the slow growth individuals, which would not have grown much larger, are considered together with the fully grown individuals.

Additional support for hypothesis (iv), strong developmental plasticity, comes from the observation that the *Plateosaurus* individuals from Frick show the same great variability in final size and growth rate as the individuals from Trossingen (Fig. 3). The individuals from Frick are smaller on average and show a smaller final size. The Frick bone bed may represent a population of generally smaller stature, possibly due to a less favorable habitat or because the Trossingen and Frick rocks have different ages.

Outgroup comparison based on long-bone histology indicates that the strong developmental plasticity is plesiomorphic for archosaurs and was retained in the crocodile lineage (*1*, *2*, *4*). Virtually all basal archosaurs and pseudosuchians have lamellar-zonal bone with numerous and distinctive LAGs (*4*). Ornithodirans (pterosaurs and dinosaurs), on the other hand, had lost developmental plasticity, as indicated by the predominance of the fibrolamellar complex (*1*, *2*, *4*, *22*, *23*). The strong developmental plasticity of *Plateosaurus* is a reversal to an ancestral condition and contrasts with all of the more derived dinosaurs and two other basal dinosaurs, the prosauropod *Massospondylus* (*7*, *24*) and the basal sauischian *Herrerasaurus* [although only two specimens of *Herrerasaurus* were sampled (*2*, *22*)]. Recent phylogenetic analyses (*15*–*18*) agree that *Massospondylus* and *Plateosaurus* are closely related, suggesting that different species of prosauropod dinosaurs varied in their degree of developmental plasticity. It may be no coincidence that a similarly unexpected reversal to an ancestral condition, i.e., quadrupedality, was recently discovered in embryos of *Massospondylus* (*25*).

In extant amniotes, strong developmental plasticity is correlated with a low metabolic rate and behavioral thermoregulation [the ectotherm strategy (*26*)], resulting in widely differing growth rates and final sizes in individuals of the same species of, e.g., turtles (*27*, *28*), lizards (*29*, *30*), and crocodiles (*31*, *32*). The observed strong developmental plasticity in *Plateosaurus* would suggest that it also was an ectotherm. This disagrees with the dominance of the fibrolamellar complex in the long-bone cortex of this dinosaur [which is not known from modern ectotherms in natural habitats and indicates high growth rates (*1*, *33*)] and its advanced dinosaurian locomotor apparatus. The early dinosaur *P. engelhardti* possibly represents the initial stage in the evolution of metabolic thermoregulation (endothermy) in dinosaurs, in which endothermy was decoupled from developmental plasticity.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5755/1800/DC1
Materials and Methods
Table S1
References

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Drought, Snails, and Large-Scale Die-Off of Southern U.S. Salt Marshes

Brian R. Silliman,[1]* Johan van de Koppel,[2] Mark D. Bertness,[3] Lee E. Stanton,[4] Irving A. Mendelssohn[4]

Salt marshes in the southeastern United States have recently experienced massive die-off, one of many examples of widespread degradation in marine and coastal ecosystems. Although intense drought is thought to be the primary cause of this die-off, we found snail grazing to be a major contributing factor. Survey of marsh die-off areas in three states revealed high-density fronts of snails on die-off edges at 11 of 12 sites. Exclusion experiments demonstrated that snails actively converted marshes to exposed mudflats. Salt addition and comparative field studies suggest that drought-induced stress and grazers acted synergistically and to varying degrees to cause initial plant death. After these disturbances, snail fronts formed on die-off edges and subsequently propagated through healthy marsh, leading to cascading vegetation loss. These results, combined with model analyses, reveal strong interactions between increasing climatic stress and grazer pressure, both potentially related to human environmental impacts, which amplify the likelihood and intensity of runaway collapse in these coastal systems.

Degradation of coastal ecosystems is occurring worldwide (*1*). Large-scale eutrophication, food web alteration, runaway consumer effects, climate change, habitat destruction, and disease have all been implicated as causes of extensive loss of key coastal ecosystems including kelp forests, mangroves, oyster reefs, seagrass beds, and coral reefs (*2*–*7*). These threats rarely occur in isolation from one another, and the realized damage we observe may well be exaggerated by synergistic interactions (as opposed to simply additive stress). Failure to understand these synergies will lead to further loss of important ecosystem services including shoreline buffering, nutrient and sediment filtering, and critical nursery and adult habitat for fisheries and shorebirds (*1*). Because near-shore communities generate >$150 billion in benefits per year through the tourism, aquaculture, and fishery industries, elucidating the complex causes of marine ecosystem degradation is one of the most pressing ecological issues of our times (*1*).

Over the past 6 years, salt marshes along the southeastern and gulf coasts of the United States experienced unprecedented die-off, with affected areas ranging in size from 100 m^2 to 3 km^2, totaling >250,000 acres, and occurring along >1500 km of coastline (*8*–*11*). These systems are the most ecologically and economically important shoreline communities along the eastern and gulf U.S. seaboard (*12*). The key to protecting these critical habitats is to understand the factors that affect marsh community structure and how those factors may be changing. For nearly 60 years, the paradigm of salt marsh ecology has been that bottom-up factors (e.g., nutrients, soil salinity) are the primary forces controlling growth of the dominant habitat-forming plant in the community, *Spartina alterniflora* (cordgrass) (*12*). Accordingly, investigations of die-off of this grass have focused on the role of physical stressors such as salinity and soil moisture–related factors (*8*–*11*).

Recent experimental evidence, however, has shown that grazing is also important in regulating grass production (*13*–*16*). Manipulation of the most abundant marsh grazer, the periwinkle snail (*Littoraria irrorata*), revealed these gastropod consumers damage live *Spartina* when grazing their fungal food (*14*, *15*). Subsequent facilitation of fungal infection in leaf tissue via snail radular activity leads to drastic reductions in aboveground plant production (*16*) and, at high densities, grazing by fungal-farming snails destroys the marsh canopy (*15*). Although both edaphic (i.e., soil-related) stresses and grazing can potentially harm salt marsh vegetation, there is currently no consensus on the relative importance of these factors in explaining marsh dieback. Extreme physical stressors [e.g., decreased soil moisture, elevated salinities in marsh soils and estuarine waters, increased soil acidity; see Table 1 (*8*–*11*, *17*)] generated by a severe drought from ~1999 to 2001 in the southern United States [Table 1, reoccurrence interval ~100 years (*17*)] and biotic interactions with fungal pathogens [*Fusarium* spp. (*11*)] have been considered as plausible causal factors (*8*–*11*). However, no studies have examined experimentally the role of grazers in either contributing to or expanding initial dieback areas.

To examine the extent and intensity of grazer impacts at marsh die-off sites, we quantified snail abundance at 12 randomly chosen die-off sites that spanned 1200 km of shoreline and experimentally investigated the relative contribution of these consumers to marsh loss at two sites in Georgia and two in Louisiana

Table 1. Rainfall levels, Palmer Drought Severity Index (PDSI) values, and salinities in Sapelo Island, Georgia (for Airport Marsh and Marsh Landing Creek) and Louisiana coastal marsh ecosystems during the peak of a 100-year drought (~1999 to 2001) event that immediately preceded marsh die-off in both states. For Georgia, drought rainfall data are for 2000 and 2001 and for predrought years from 1958 to 1999. The PDSI, which is a meteorological drought index based on precipitation, temperature, and available water content of the soil, ranges from 4.0 or more for extremely wet conditions to –4.0 or less for extreme drought and covered the same time periods as for rainfall. For salinities, data are averages of monthly (soil salinity) and daily (estuarine salinity) measurements taken at the same location on Sapelo Island, Georgia, from June to September in 2000 and 2001 and for nondrought years from June to September in 2003 and 2004 (mean 2003–2004 rainfall = 134.4 cm). For Louisiana, drought rainfall is for 1999 and 2000 and for predrought years from 1950 to 1998. The PDSI is provided for the same time periods as for rainfall. Estuarine salinities before the drought were for the period from 1961 to 1998; drought estuarine salinities were for 1999 and 2000. For Louisiana, data are averages of monthly means. Data are means ± SD.

Drought indicator	Georgia		Louisiana*	
	Drought	Predrought/ nondrought	Drought	Predrought
Yearly rainfall (cm)	93 ± 7.5	131 ± 21	102 ± 76	132 ± 74
PDSI	–2.71 ± 0.57	0.18 ± 1.56	–2.85 ± 1.27†	0.11 ± 2.02†
Estuarine salinities (ppt)	30.5 ± 1.41	20.8 ± 3.54	25.5 ± 0.6	20.6 ± 5.6
Marsh soil salinities (ppt)	48.6 ± 4.27	27.3 ± 2.58	†	†

*Data modified from (*22*). †Grand Terre, LA. Although interstitial salinities before and after the drought are not available for Louisiana, interstitial salinities collected in June 2000 at a dieback site at Bay Junop ranged from 27 to 32 ppt; salinity was 23 ppt in an adjacent nondieback site (*8*).

[1]Department of Zoology, University of Florida, Gainesville, FL 32611, USA. [2]Centre for Estuarine and Marine Ecology, Netherlands Institute of Ecology (NIOO-KNAW), NL 4401 NT7 Yerseke, Netherlands. [3]Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912, USA. [4]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803, USA.

*To whom correspondence should be addressed. E-mail: brs@ufl.edu

Table 2. Snail densities (individuals/m^2) at 12 marsh die-off sites in the southeastern United States. In the summer of 2002, at each site, 10 quadrats (50 cm by 50 cm) were randomly placed (i) along the two types of die-off border (i.e., the exposed mudflat-healthy marsh interface) typically encountered at each site—that is, borders with high snail densities and those with low densities; (ii) on exposed mudflats; and (iii) in adjacent healthy marsh areas. Lengths of high-density snail fronts are in parentheses. Data are means ± SD.

Site	Snail density			
	Exposed mudflat	Die-off border with low snail density	Die-off border with high snail density	Healthy marsh
Bourbon Field Marsh, Sapelo Island, GA	0	223 ± 96	1076 ± 243 (98 m)	176 ± 59
Airport Marsh, Sapelo Island, GA	0	564 ± 132	2175 ± 675 (68 m)	487 ± 92
Dean Creek, Sapelo Island, GA	1.2 ± 2.3	48 ± 26	416 ± 45 (24 m)	185 ± 67
Lighthouse Marsh, Sapelo Island, GA	0	834 ± 112	2634 ± 456 (128 m)	558 ± 121
South Marsh, Ossabaw Island, GA	0	114 ± 43	457 ± 167 (32 m)	78 ± 17
Jerico River, Bryan County, GA	0	3 ± 3	15 ± 9	1.4 ± 0.8
South Marsh, Talahi Island, GA	0	128 ± 87	412 ± 254 (14 m)	68 ± 11
Exp. Site 1, Fort Fourchon, LA	0	67 ± 32	1356 ± 387 (212 m)	82.2 ± 12.6
Exp. Site 2, Fort Fourchon, LA	0.2 ± 0.1	45 ± 29	1067 ± 365 (185 m)	68.4 ± 14.6
Watch Tower Marsh, Bell Buruch, SC	0	38 ± 16	225 ± 112 (13 m)	49 ± 15
Inlet Marsh, Bell Buruch, SC	0	63 ± 31	315 ± 96 (13 m)	56 ± 17
Charleston, SC	0	32 ± 18	402 ± 167 (22 m)	96 ± 37

(*17*). An initial survey of dieback sites revealed extreme densities of plant-grazing snails, commonly 500 to 2000 individuals/m^2, aggregated in extensive fronts on die-off borders. Snail density was near zero on exposed mudflats, peaked on the die-off/healthy marsh border, and dropped off considerably within healthy marsh (Table 2).

To test the hypothesis that *Littoraria* grazing expands marsh dieback areas, we placed 1-m by 1-m wire-mesh exclosures in July 2002 (i) just ahead of fronts of snails on die-off borders, and (ii) in remnant *Spartina* patches inside die-off areas. We monitored the effects of snail removal on cordgrass biomass and soil physical conditions for ~14 months until September 2003 (*17*). We also examined the effects of snail density on expansion of die-off border and quantified the total vegetated area cleared by moving consumer fronts (*17*). Finally, to examine the relationship between both consumer front persistence and propagation and snail density in adjacent healthy areas, we set up permanent 50-cm by 50-cm plots along line transects running perpendicular to die-off borders (–5 m, inside die-off area on exposed mudflats; 0 m, on border; 5 m, within healthy areas; 10 m; and 20 m) and monitored snail densities and *Spartina* biomass in those plots over a 1-year period starting in July 2002 (*17*).

Recent studies have suggested that top-down control by fungal-farming snails intensifies when *Spartina* is either stressed or receives nutrient enhancement, because facilitated, facultative pathogenic fungi growing in grazer wounds on live leaves experience enhanced growth (*14*–*16*). To test whether sublethal drought stress observed in marsh soils and estuarine waters throughout the Southeast (*8*–*11*, *17*) (Table 1) could have acted together with grazing to generate initial die-off areas, we experimentally elevated salt concentrations in marsh soils with and without snails at one healthy marsh site in Georgia (434 ± 54 snails/m^2) from March to October 2002 (*17*).

Experimental manipulation of *Littoraria* on high-density borders and in remnant *Spartina* patches supports our hypothesis that snail grazing contributes to expanding dieback in southern U.S. salt marshes (Fig. 1). In both Georgia and Louisiana marshes, snail removal increased *Spartina* biomass by more than three orders of magnitude, whereas continued snail access to *Spartina* in control plots resulted in heavy overgrazing, destruction of marsh canopy, and conversion of healthy salt marsh cordgrass to exposed mudflat both along die-off borders and on edges of remnant *Spartina* patches (Figs. 1 and 2). The primary mechanism by which snails killed *Spartina* was likely through facilitation of pathogenic fungi during farming activities—an indirect pathway of control, as has been shown in past studies (*14*, *16*)—and not through consumption of live plant material. In all plots, there was no evidence that physical stress negatively affected *Spartina* health (*17*), as caged, isolated patches of cordgrass on mudflats (Fig. 2) were dense, robust, and green in the absence of intense snail grazing. This observation of minimal soil stress during our caging study (July 2002 to July 2003) is consistent with the cessation of drought conditions in Georgia and Louisiana marshes in 2002 (Table 1). Combined with results from snail exclusion experiments (Fig. 1), these findings imply that runaway consumption can carry on the process of vegetation loss in the absence of drought-induced effects.

After moving through control plots, snail fronts maintained structure and continued to eliminate healthy marsh (Fig. 1). As concentrated waves of consumers passed over vegetated areas, *Spartina* grass disappeared and exposed mudflats remained (Figs. 2 and 3). How far and how long these fronts propagated, however, was strongly dependent on snail density both in the front itself and at the experimental sites before die-off (*17*). When snail density in consumer fronts was high (>1000 individuals/m^2), front propagation from initially marked areas through healthy marsh was extensive, reaching a maximum of 14 m in Louisiana and 31 m in Georgia, whereas at lower densities (100 to 400 individuals/m^2) front effects were significantly diminished (Fig. 1). From a temporal perspective, when snail densities before marsh die-off and outside of fronts were low, as in Louisiana (78 ± 33 individuals/m^2), snail fronts broke up in <8 months. In contrast, at Georgia sites, where snail densities outside of fronts and before die-off were high (485 ± 124 individuals/m^2), front propagation lasted >1 year (Fig. 3). The tendency for consumer fronts to break up and for snails to spread out likely occurs because *Littoraria* experience intense intraspecific competition at high densities (*14*–*16*).

The total extent of marsh destroyed by snails (*17*) tracked background snail density and front persistence. Snail grazing in two Louisiana marshes resulted in relatively small but significant expansion of die-off areas (an 11% increase, from 22,980 m^2 to 25,320 m^2). In Georgia, long-lived snail fronts converted extensive marsh habitat into exposed mudflat, increasing original die-off areas by at least 185% (from 2230 m^2 to 6456 m^2; because snail fronts were already formed when we arrived, total impact estimates are underestimations). Thus, when grazer density is high, marsh systems become more susceptible to runaway grazing, and top-down forces can be responsible for significantly larger proportions of marsh die-off. Our survey of 12 marshes in the southeastern United

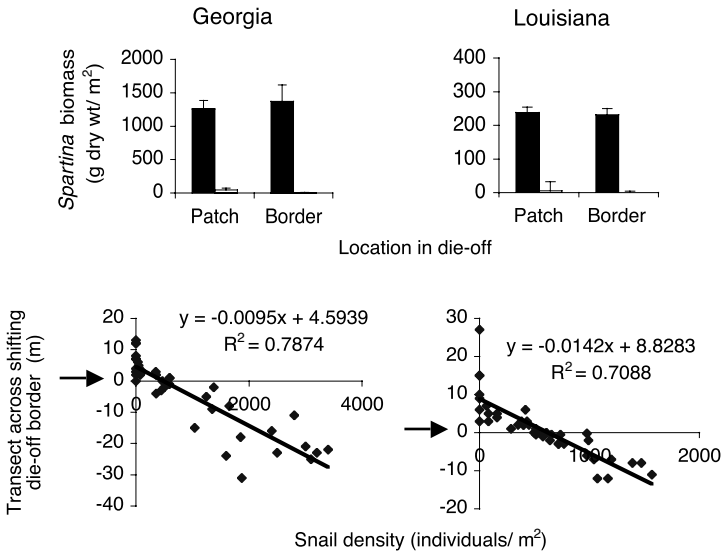

Fig. 1. Effects of snail exclusions on *Spartina* biomass in interior (i.e., remnant patches of live cordgrass) and on border of marsh die-off sites in Georgia (left) and Louisiana (right), and least squares linear regression of mean *Littoraria* density on 5-m sections of die-off border and net movement of that border over 1 year in Georgia and Louisiana (n = 39 for Georgia, n = 44 for Louisiana). In the lower panels, 0 m on the line of the abscissa indicates original position of die-off border and is denoted by a horizontal arrow; negative numbers close to the current 0-m position indicate further marsh loss and expansion of mudflats, whereas positive numbers indicate community recovery and grass growth into die-off areas. For the caging experiment, data for each state were separately analyzed using a two-way (treatment × site) analysis of variance (ANOVA). Data either exhibited homogeneity of variance and were normally distributed or were transformed using log transformations for assumption conformity. Only linear contrasts were compared, using Tukey's post hoc test. Because we found no significant effect of site (P > 0.43, both cases), data from replicate sites were pooled. Solid bars (left) indicate snail exclusion treatments; open bars (right) indicate uncaged controls. Error bars indicate SE.

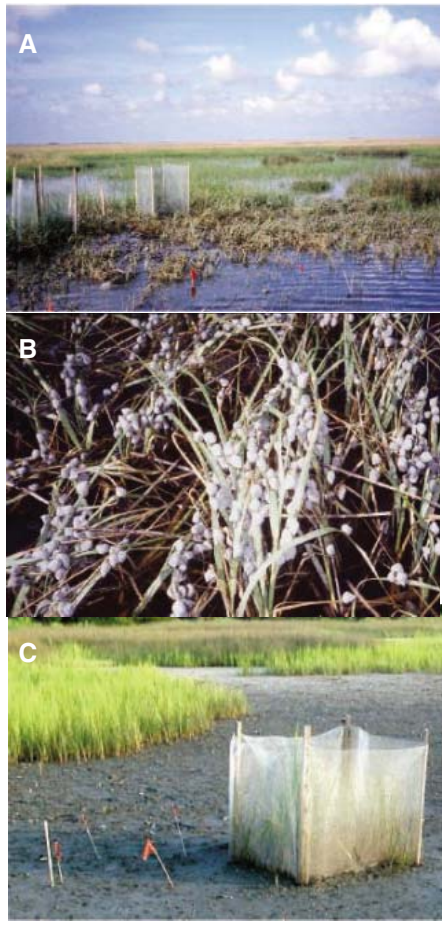

Fig. 2. (**A**) Snail front exclosures (wrapped in hardware cloth) installed on die-off border at one site in Louisiana. Gray area in front of cages is a snail front. (**B**) Representative extreme snail densities found in snail front pictured in (A). (**C**) Effect of exclusion cage on *S. alterniflora* biomass on die-off border at the Lighthouse Marsh in Georgia.

States (Table 2) revealed snail fronts at 11 of 12 die-off areas, which suggests that consumer wave propagation was an important, yet overlooked (*8*–*11*), contributing factor to the expansion of dieback throughout the Southeast.

Reconstruction of snail densities before marsh die-off and severe drought conditions from 1999 to 2001 (Table 1) (*8*–*11*) indicate that snail grazing alone did not initiate marsh die-off in Louisiana or Georgia. In Louisiana, snail densities [78 individuals/m^2 (*14*, *15*)] were well below levels needed to completely denude vegetated marsh under nonstressful conditions, and all evidence to date points to drought-induced soil moisture stress and related edaphic factors (e.g., salinity, acidification, and metal toxicity) as the primary initiating factors (Table 1) (*8*–*11*, *17*). However, only nonlethal physical conditions were observed in the field, both during die-off events in Louisiana and Georgia (Table 1) and afterward (*8*–*11*). These data suggest that less intense, sublethal drought stress interacting with snail grazing could also have caused or contributed to initial marsh die-off in some instances. In Georgia, die-off occurred extensively at many locations with low snail density (Jerico River marsh, Table 2) (*9*, *10*), but at many sites in Georgia snail densities were high enough (100 to 600 individuals/m^2, Table 2) to strongly suppress *Spartina* growth (*14*, *15*) and potentially interact synergistically with drought-induced stress.

Our experimental simulation of drought-induced salt stress supports this prediction and reveals that elevated salinities intensify top-down control by snails. Experimentally increasing dissolved salt concentrations in the soil to approximate drought conditions [from 35.33 ± 2.63 parts per thousand (ppt) to 56.83 ± 6.24 ppt; see Table 1 for comparisons] in the absence of snails reduced grass growth by 45%, whereas in the presence of snails, high salt levels had a significantly greater negative effect (84% biomass reduction), resulting in near-complete mortality of *Spartina* (*17*). Thus, although drought stress was likely the overwhelming cause of initial die-off when snail densities were low [most Louisiana marshes; Table 2 (*8*–*11*)], our experimental results and surveys indicate that climate-induced stress and consumer pressure acted together at many other sites (i.e., in marshes with high snail density in Georgia) to generate original die-off disturbances.

On the basis of this analysis and our experimental results, we hypothesize that extensive die-off in southern marshes was caused by the following sequence of interacting events: (i) Protracted and intense drought (3 to 4 years) resulted in stressful soil conditions, which acted either alone or in combination with plant-grazing snails to cause rapid community die-off in well-defined, distinct areas. (ii) After these areas were denuded by grazers and natural processes, predictable, behaviorally driven snail movement toward live *Spartina* (*17*) led to snails concentrating on die-off borders, forming consumer fronts. (iii) Runaway grazing and density-dependent propagation of snail fronts, which persisted >1 year after drought impacts subsided, carried on the process of vegetation loss to expand the total area of marsh degradation. After drought stress subsided in 2002, some die-off areas began to recover in 2003 via vegetative growth from adjacent healthy marsh (*17*), but only along borders without snail fronts (Fig. 1). To date, most die-off areas have yet to fully recover (*17*), but the trend for marsh plants to recolonize exposed mudflats and for consumer fronts to break up shows that vegetation loss in this system is not unidirectional for the observed levels of grazer density and drought-induced stress.

We developed a spatially explicit model that supports our experimental findings, indi-

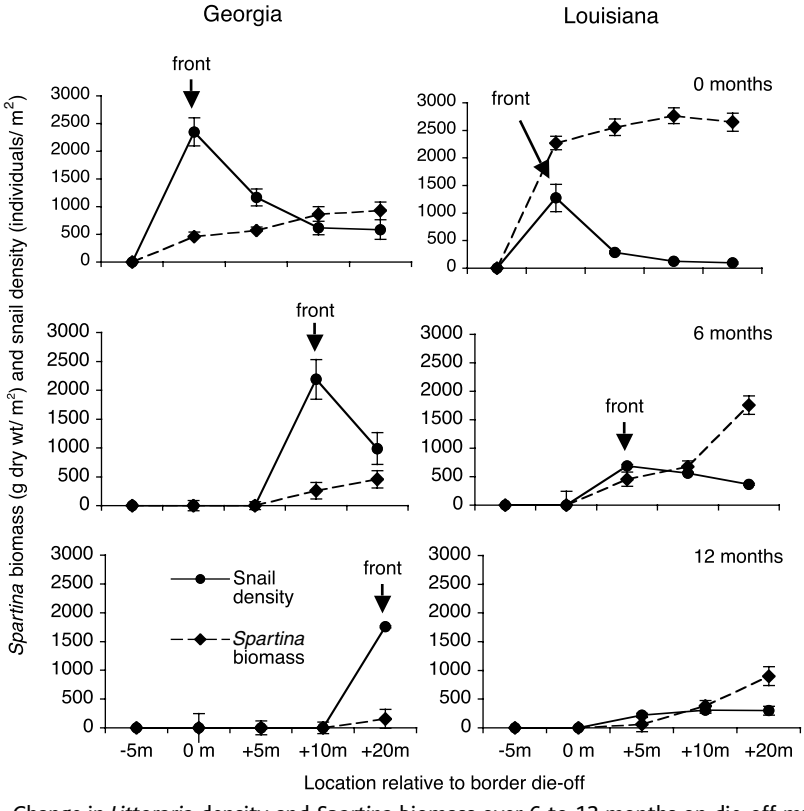

Fig. 3. Change in *Littoraria* density and *Spartina* biomass over 6 to 12 months on die-off mudflat (–5 m), original border at time zero (0 m), and 5, 10, and 20 m into live marsh at two die-off sites in Georgia (left) and Louisiana (right). For each distance at each site, $n = 5$. In Georgia and Louisiana, readings were taken at time zero (July 2002), ~6 months later, and after ~1 year. Differences in snail density and *Spartina* biomass were assessed separately for each state and date using a two-way (distance × site) ANOVA. We did not use repeated-measures analysis or Bonferroni adjustments because we had a priori hypotheses about each date (i.e., snail fronts would be moving toward grass, dissipating and mowing down *Spartina*). Because there was no site effect ($P > 0.16$), we pooled data from replicate sites for one analysis per state per date. For snail densities, there was a significant effect of distance in all cases ($P < 0.02$). For *Spartina* biomass, there was a significant effect of distance at time zero and after 6 and 12 months in Louisiana ($P < 0.04$) and for time zero and after 6 months in Georgia ($P < 0.001$).

cating that (i) the interaction of drought stress, grazing, and directional snail movements can lead to formation of traveling consumer waves (*17*) (fig. S1A), and (ii) consumer fronts form only when both edaphic stress and snail densities are high, but not when either of these factors occurs in isolation (fig. S1B). In addition, model analyses point to the multiplicative effects of two forces that have the potential to strongly affect the health of southeastern U.S. marshes: (i) increased incidence of intense drought, caused by increasing climatic extremes [see discussion in (*8*)], and (ii) decreased snail mortality rates, which have the potential to occur as a result of recent population declines in blue crabs [40 to 85% (*18*)], a major predator of *Littoraria* (*15*). Occurring simultaneously, these factors could act synergistically to increase system susceptibility to even more intense and prolonged vegetation loss and, potentially, unidirectional large-scale collapse. Increased drought stress lowers the threshold grazer density needed to generate consumer fronts and runaway consumption of *Spartina*, as we have shown, while decreased snail predation may allow that critical threshold to be crossed even faster. Although decreased predation by blue crabs could lead to lowered snail mortality rates, more study is required to clarify the sources of snail predation and human influence on top-down control in this system.

Our experimental results provide definitive field evidence that the interplay between physicochemical stressors and trophic interactions plays an important role in die-off of coastal salt marshes in the southern United States. Drought-induced soil stress can amplify top-down control by grazers and initiate marsh plant die-off in localized intertidal areas. These disturbances then stimulate the formation of consumer fronts, leading to waves of salt marsh destruction resulting from runaway consumption. These findings stress that interactions between food web dynamics and climate must be considered when investigating community collapse in coastal systems. Despite similar observations of consumer fronts leading to habitat collapse in kelp beds [sea urchins (*2*)], arctic salt marshes [snow geese (*19*)], and coral reefs [sea stars (*20*)]—all initially in response to human alteration of food web structure or nutrient regimes [but see (*21*) for evidence of emerging climatic feedbacks in arctic marshes]—there is relatively little understanding of how the potential for runaway consumption will interact with climate change–induced weather extremes to affect system stability and persistence. Recognition of the interactive, nonlinear processes by which such effects are manifested is paramount for successful management of ecosystems that face anthropogenic impacts through multiple stressors.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5755/1803/DC1
Materials and Methods
Fig. S1
References

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Island Biogeography of Populations: An Introduced Species Transforms Survival Patterns

Thomas W. Schoener,[1]* Jonathan B. Losos,[2] David A. Spiller[1]

Population phenomena, which provide much of the underlying basis for the theoretical structure of island biogeography, have received little direct study. We determined a key population trait—survival—in the Bahamian lizard *Anolis sagrei* on islands with an experimentally introduced predatory lizard and on neighboring unmanipulated islands. On unmanipulated islands, survival declined with several variables, most notably vegetation height: The island with the shortest vegetation had nearly the highest survival recorded for any lizard. On islands with the introduced predator, which forages mostly on the ground, *A. sagrei* shifted to taller vegetation; unlike on unmanipulated islands, its survival was very low on islands with the shortest vegetation but was higher on the others. Thus, species introduction radically changed a resident species' relation of survival to a key island-biogeographical variable.

When MacArthur and Wilson first proposed their highly influential equilibrium theory of island biogeography (*1*), a main objective was to predict patterns of species richness as a function of island area and distance. Subsequent research broadened the island properties to include variables such as habitat and elevation, but the focus on understanding species richness and related community characteristics remained (*2*–*6*). Likewise, experimental approaches to island biogeography dealt almost entirely with community rather than with population aspects (*7*). However, the equilibrium theory and its derivatives rely to a great extent on population phenomena for their underlying assumptions, making research on population traits necessary for mechanistic understanding. Here, we report an experimental study designed to examine how a prey population-level trait, in this case survival, relates to major island variables. We investigated not only island area, an original component of the island-equilibrium model, but also a habitat variable—vegetation height—because a number of previous studies have shown that such variables were better predictors of species richness than area. We also investigated a key ecological variable, population density of the subject species, the lizard *Anolis sagrei*.

We selected 12 neighboring islands varying in area, habitat, and *A. sagrei* population size (population size and population density are strongly correlated: $r = 0.86$). The islands are located near Snake Cay and Buckaroon Bay, Abaco, Bahamas. The islands range from 104 to 324 m^2 in vegetated area, at the small end of the continuum for islands that have *A. sagrei* (*8*). They are rather sparsely covered with trees (up to 3.8 m in height) and/or shrubs (many quite prostrate). On six islands, we introduced the larger lizard *Leiocephalus carinatus*, a predator on the smaller *A. sagrei*. To avoid strong predator density dependence, *L. carinatus* propagule size was scaled to the population size of *A. sagrei* (*9*). The introduction of the larger lizard caused *A. sagrei* to shift to higher perches (*10*). As the islands differed substantially in the availability of such perches, we selected a measure of vegetation height as the most relevant habitat variable.

The unmanipulated islands, containing *A. sagrei* with no introduced predator, showed negative relations of survival (percentage of the original cohort still alive by Date X) to all three variables (*9*). After 6 months (the time when about half the *A. sagrei* individuals had died), Pearson r for vegetation height was –0.90 (using the arcsin square-root transformation for survival and log transformation for height) and r values for *A. sagrei* population density and area (both log-transformed) were –0.75 and –0.37, respectively. Annual survival (*9*), highly related to the 6-month measure, gives similar results for vegetation ($r = –0.86$), density ($r = –0.81$), and area ($r = –0.28$). If we focused on a single one of these correlations, a Pearson r exceeding in magnitude –0.73 would be significant at the 5% level (one-tailed P). However, the results are highly related because the three island variables are positively intercorrelated: area/density $r = 0.34$, area/vegetation height $r = 0.55$, and vegetation height/density $r = 0.61$ (Fig. 1). Two of the variables are statistically confounded, and collinearity measured by the condition number is much too high (*11*) to perform multiple regression of survival with any two or all three island variables (*9*). Rather, we accept as the best description the single island variable giving the strongest correlation with survival—namely, vegetation height (Fig. 2). Survival values over the six islands span the great majority of the possible range: Survival after 6 months varies from the nearly immortal (91.4% of individuals survived on the island that had the shortest vegetation, which was also the smallest island) to a very high mortality (25.6% of individuals survived on the island that had the tallest vegetation, which was also the largest island). Annual survival on the island with the shortest vegetation is still 80%, tied for the highest determined for any *Anolis* and only exceeded by one other lizard species population known to us (*12*).

Notably, vegetation height gives a substantially stronger correlation with survival than does area. Islands with higher vegetation are especially attractive to birds: A study of more than 500 islands in the Bahamas showed that occurrences and diversity of bird species in-

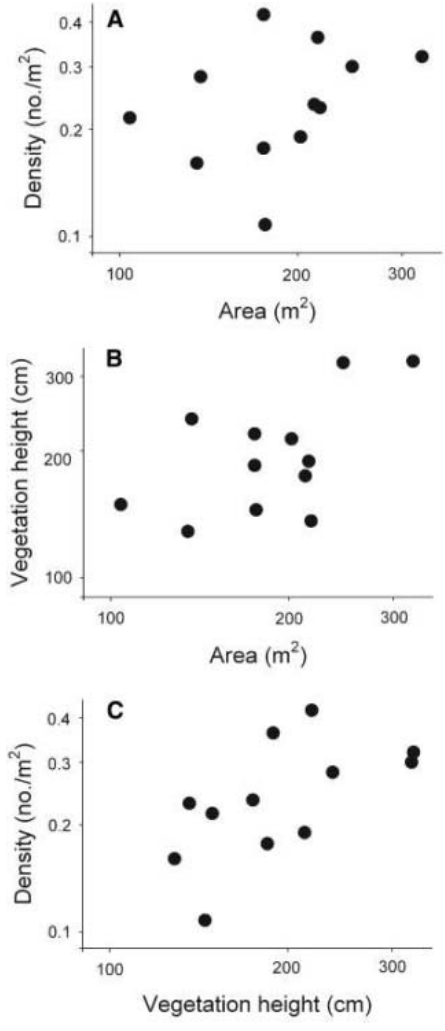

Fig. 1. Relationships between variables characterizing the 12 study islands. (**A**) *A. sagrei* population density versus area. (**B**) Vegetation height versus area. (**C**) Population density versus vegetation height.

[1]Section of Evolution and Ecology, University of California, Davis, CA 95616, USA. [2]Department of Biology, Washington University, St. Louis, MO 63130, USA.

*To whom correspondence should be addressed. E-mail: twschoener@ucdavis.edu

crease more with amount of higher vegetation than with other variables (*8*), including area. We would therefore expect this result if birds were the main natural predator on our 12 study islands. Prey density, however, is also strongly correlated with survival, which also suggests bird predation, particularly from itinerants. The larger the density of prey on an island, the more likely itinerant predators will be present, because it is profitable to spend more time there (*13*). This expectation is often supported by data (*14*). Furthermore, the greater the density, the more per capita interaction there is among prey individuals, leading to a greater likelihood of capture by predators (*15*).

How does the addition of a large predatory lizard species change this marked relation of survival to island vegetation height? The predator is substantially longer and stockier than *A. sagrei* and perches mainly on the ground and rocks. It only occasionally (and sometimes awkwardly) perches on the vegetation, but generally close to the ground and on relatively thick branches. Hence, a refuge from this predator for *A. sagrei* is the vegetation, particularly the higher branches and twigs (which tend to be thinner). One might expect, therefore, that availability of such perches would enhance survival in *A. sagrei* (*16*). If no other factors were involved, this would imply that islands with higher vegetation should have higher survival, the opposite of the trend expected from the other (e.g., bird) predators, which was indeed found on unmanipulated islands. As expected, results after introducing the large terrestrial predator give very different plots of survival versus vegetation height for the six manipulated islands than for the controls. First, the relation is now positive, not negative as it is for the unmanipulated islands (Fig. 2). Pearson r values are high but not quite statistically significant at the 5% level (6 months, $r = 0.65$; 1 year, $r = 0.71$). However, an analysis of covariance on the entire set of 12 islands gives a highly significant (treatment)*(vegetation height) interaction term (two-tailed $P = 0.005$ for both 6 months and 1 year). Second, the shapes of the curve are different (supporting online material text).

The difference in survival values between islands with and without the introduced predator varies substantially with vegetation height (Fig. 2). For islands with the shortest vegetation (which tend to be small), survival is much greater in the absence of the introduced predator. In particular, 80.0 to 91.4% of lizards on the two control islands with the shortest vegetation survived 6 months, whereas 6.3 to 15.2% did so on the two introduction islands that had the shortest vegetation (values of vegetation height are very similar between controls and introductions). In contrast, for moderate to large vegetation heights, little to no difference in survival exists; if mortality factors were additive, one might expect that introduction islands with vegetation of these heights would have lower survival. Instead, the pattern found is consistent with compensatory mortality factors (*17*–*19*); no more lizards were killed on islands with moderate to large vegetation height in the presence of the introduced predator than were killed in its absence.

How might such compensation take place? A simple explanation is that mortality from predators other than *L. carinatus* is smaller on introduction than on control islands. But why would mortality from other predators decline? It seems likely that such predators would be mostly itinerant birds (rats are also possible), which include the study islands within their foraging range but only occasionally (if ever) nest on one of them. Were potential prey reduced by the introduced lizards on a particular island, such itinerant predators would feed there less, much as is depicted by optimal foraging conceptualizations (*13*). In other words, the predator that can select feeding sites over a broad region can avoid those islands to which the nonitinerant introduced predators are necessarily confined (*20*). Additionally, especially vulnerable prey individuals will mostly be eaten by the introduced lizard predator, leaving few prey that the itinerant predators can easily capture.

Losey and Denno (*17*) showed for an arthropod assemblage that when a predator caused a shift of prey to a different habitat, a synergistic effect arose in which the prey suffered from the predators in the original habitat and were more vulnerable to the predators in the new habitat. In this case, not only was compensatory predation not operating, but even additive predation underestimated the combined effect of the several predators. A similar phenomenon might have been expected for our system, with *A. sagrei* shifting upwards in the vegetation and thereby increasing their vulnerability to predation by birds. However, the pattern of survival that we found is inconsistent with this expectation, leading to the inference that predation by birds takes place mostly at lower sites, including the ground, perhaps because *A. sagrei* is in general much more vulnerable there. A trial (*9*) in which clay-model lizards were placed in low positions (on the ground or rocks) produced significantly fewer marks—plausibly the result of bird pecking (*21*, *22*)—on the control island with the second-shortest vegetation (13%) than on the control island with the tallest vegetation (67%; $P = 0.007$). The difference is in line with Fig. 2 and implies substantial attempted predation at low sites on the nonintroduction island with the tallest vegetation.

The kind of compensation we are hypothesizing can provide the basis of a simple model giving the form (*9*) and positioning of the curve relating survival to vegetation height on introduction islands. Assume that itinerant bird

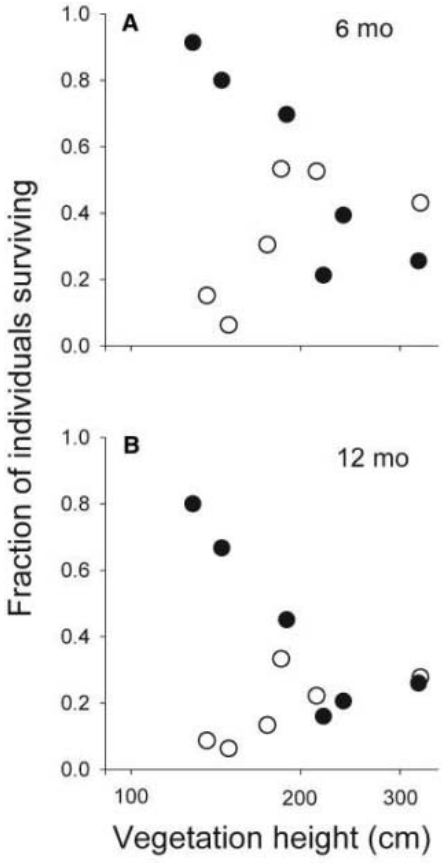

Fig. 2. Survival fractions versus vegetation height on control (solid) and introduction (clear) islands in November 2003, ~6 months after initiation of the experiment (**A**) and in May 2004, ~1 year after initiation of the experiment (**B**).

predators kill those prey not killed by the introduced lizard predator, but only up to the point that the number they kill plus the number the introduced lizard predator kills equals the number the itinerants would kill if the island did not have the introduced lizard predator. The rule is roughly consistent with the marginal value theorem (*13*), whereby all patches (regardless of the value when entered by the predator) are left at the same marginal intake rate, corresponding to the same giving-up density (*23*). Such marginals are unlikely to change much for the entire site by manipulation of six islands, so the departure rule should be about the same with and without the introduced predator. Thus, on islands where the introduced predator kills a relatively large percentage of the prey, itinerants would kill no additional prey. On islands where prey are better able to avoid the introduced predator, itinerants kill some prey, but fewer than they would in the absence of the introduced predator. Figure 3 gives hypothetical examples. Assume (Fig. 3A) that the percentage of prey killed on control islands by itinerant birds rises linearly with vegetation height and then asymptotes, approximately as the data for control islands behave (Fig. 2). The curve representing

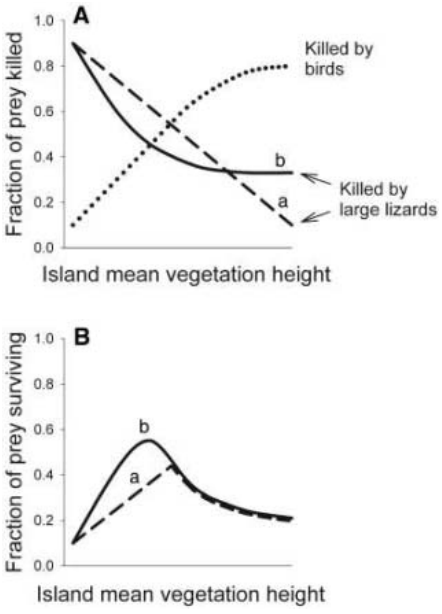

Fig. 3. (**A**) Hypothetical mortality curves as a function of vegetation height. The fraction of prey killed by the large introduced lizard predator decreases with vegetation height, either linearly (a) or asymptotically (b). The fraction of prey killed by natural predators such as itinerant birds increases with vegetation height, with a small asymptotic portion at the end. (**B**) Predicted survival curves as a function of vegetation height. These curves consist of two segments, the first of which is the complement (1 – fraction killed) of the large lizard predator curve to the left of the intersection, and the second is the complement of the itinerant bird predator curve to the right of the intersection.

predation by the large lizard is assumed to decline monotonically with vegetation height, either linearly (curve A) or asymptotically (curve B). The actual number killed by both predators is then the number killed by the introduced large lizard or the number killed by itinerant birds on the control island, whichever is larger. The resulting survival curves for introduction islands are depicted in Fig. 3B, which should be compared with the actual curves of Fig. 2. Although consistent with the data and hypothesized mechanisms involving itinerant birds, definitive evaluation awaits further investigation.

By using an archipelago of small islands as a laboratory for both comparative study and manipulative experiment, we showed a notable natural relation of survival rate to island characteristics—especially vegetation height—and the ability of an introduced predator to transform that relation. Implications of this study extend beyond islands: Vegetation structure over much of Earth's surface is being precisely characterized, in part to understand how species populations respond to anthropogenic changes in land use or in climate (*24*–*26*). However, information beyond vegetation structure may be crucial. Thus, in our study, survival is unrelated to vegetation height if data from all 12 islands are considered together (r = –0.23, –0.24), yet knowing which islands have the introduced predator makes the latter a good predictor of survival rates.

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15. Deleterious effects increasing with density that are purely intraspecific are also possible. For the subject *A. sagrei*, it is unlikely that such effects—intraspecific competition for food and consequences of aggressive interactions—would by themselves result in mortality, because the lizards marked for this study were relatively large.
16. Moreover, *L. carinatus* is thermophilic, depending on sufficient sunlight for its activity during cooler times of the year. Islands having taller vegetation on average have less sunlight striking the ground, which is where *L. carinatus* mostly forage. Hence, their average predation rate should be lower on such islands.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5755/1807/DC1
Materials and Methods
SOM Text
References

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Long-Term Modulation of Electrical Synapses in the Mammalian Thalamus

Carole E. Landisman[1,2]* and Barry W. Connors[1]

Electrical synapses are common between inhibitory neurons in the mammalian thalamus and neocortex. Synaptic modulation, which allows flexibility of communication between neurons, has been studied extensively at chemical synapses, but modulation of electrical synapses in the mammalian brain has barely been examined. We found that the activation of metabotropic glutamate receptors, via endogenous neurotransmitter or by agonist, causes long-term reduction of electrical synapse strength between the inhibitory neurons of the rat thalamic reticular nucleus.

Connexin36 (Cx36)-containing gap junctions are a major mechanism of communication between the inhibitory neurons of the rodent thalamic reticular nucleus (TRN) (*1*, *2*). More than 50% of neighboring TRN neurons interact via electrical coupling. TRN neurons, which provide feedback inhibition to the thalamus, also receive strong glutamatergic synaptic inputs from neurons in the deep layers of neocortex (*3*). These corticothalamic fibers can activate metabotropic glutamate receptors (mGluRs) on TRN neurons (*2*, *4*–*7*).

We used dual whole-cell recordings in rat thalamocortical slices under infrared–differential interference contrast (IR-DIC) visualization (Fig. 1A) to measure the strength of electrical synapses interconnecting adjacent neuron pairs [coupling coefficient (cc) = 0.08 ± 0.06, mean ± SE, n = 30 pairs]. The strength of electrical coupling was tested before and after briefly tetanizing corticothalamic (CT) fibers or applying the mGluR agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic

[1]Department of Neuroscience, Division of Biology and Medicine, Brown University, Providence, RI 02912, USA. [2]Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, GB2-504, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: Carole_Landisman@hms.harvard.edu

acid (ACPD). Ionotropic glutamatergic responses were blocked pharmacologically in all experiments.

The tetani depolarized 15 of 18 neurons tested (from nine paired recordings) (Fig. 1B); of these, 11 fired action potentials, and the remaining 3 neurons hyperpolarized in response to the stimuli. All cells recovered their baseline resting potential (V_{rest}) within 500 ms to 2 s poststimulus. Changes of coupling strength were tested after baseline recovery.

Tetanic stimulation caused a long-lasting reduction in electrical coupling strength. In one example, the coupling coefficient was reduced by 36% after the tetanus and showed no signs of recovery, even 40 min posttetanus (Fig. 1C). More detailed analysis of the time course of coupling before and after the tetanus revealed that prestimulus coupling strength varied little, but within 5 min after CT shocks the coupling coefficient dropped to steady state values, where it remained for the duration of most recordings (Fig. 1D). Both the pretetanus stability and the long-term changes in coupling strength after stimulation were found for all pairs tested (n = 9). However, two of the nine pairs showed slight recovery of coupling strength ≥ 30 min poststimulus, and one pair showed full recovery at 20 min. Overall, the coupling coefficient (cc) and the junctional conductance (Gc) (*8*, *9*) of the electrical synapses dropped for all 9 pairs (cc mean = –24% and range from –6 to –52%; Gc mean = –19% and range from –9 to –36%) (Fig. 1E). A small consistent drop caused the difference in Gc versus cc reduction in input resistance (R_{in}) after tetanic stimulation (12 of 18 cells from nine pairs), but this change was not significant (mean ± SE change = –7 ± 6%, n = 18 cells, P = 0.27, two-tailed paired t test). The tetanus-induced changes in coupling strength were prevented by including an mGluR antagonist in the bath before stimulation (Fig. 1F).

We have previously shown that TRN responses to tetanic stimulation can be simulated by application of ACPD (*2*) (fig. S1). The mGluR agonist depolarized all cells and induced continuous spiking (Fig. 2A). Three to five min after washout, resting membrane potential returned to baseline levels. Over a population of cells, there was no consistent increase or decrease in R_{in}; however, most cells did exhibit immediate changes in R_{in} (both increases and decreases) with ACPD in the bath (mean change ≤ 5 min of ACPD start = 0 ± 15%, n = 9 cells). R_{in} remained stable and similar to predrug amounts for at least 10 to 30 min post-ACPD (mean change of –7 ± 17%, P = 0.11, two-tailed paired t test). During control recordings of similar duration but without ACPD application, membrane R_{in} was stable.

ACPD reduced electrical coupling responses of TRN cells (Fig. 2B) to a degree comparable to the effects of CT tetanus. During a current step applied to TRN 1, the amplitude of the postsynaptic voltage in TRN 2 dropped 23% after ACPD application, indicating a reduction in coupling strength. The weakened response persisted after washout. All neuron pairs tested showed a decrease in coupling coefficient and coupling conductance after ACPD application [cc = –26 ± 5% and Gc = –23 ± 3% (mean reduction ± SE), n = 7 pairs) (Fig. 2E). The pooled data were similar to the single case shown (Fig. 2C) and demonstrated that 25 min of washout did not restore predrug coupling strength (Fig. 2E). As seen with the tetanus paradigm, prior application of (S)-α-methyl-4-carboxyphenylglycine (MCPG) prevented ACPD-induced changes for all pairs tested, indicating that the change in coupling strength required mGluR activation (Fig. 2, D and F).

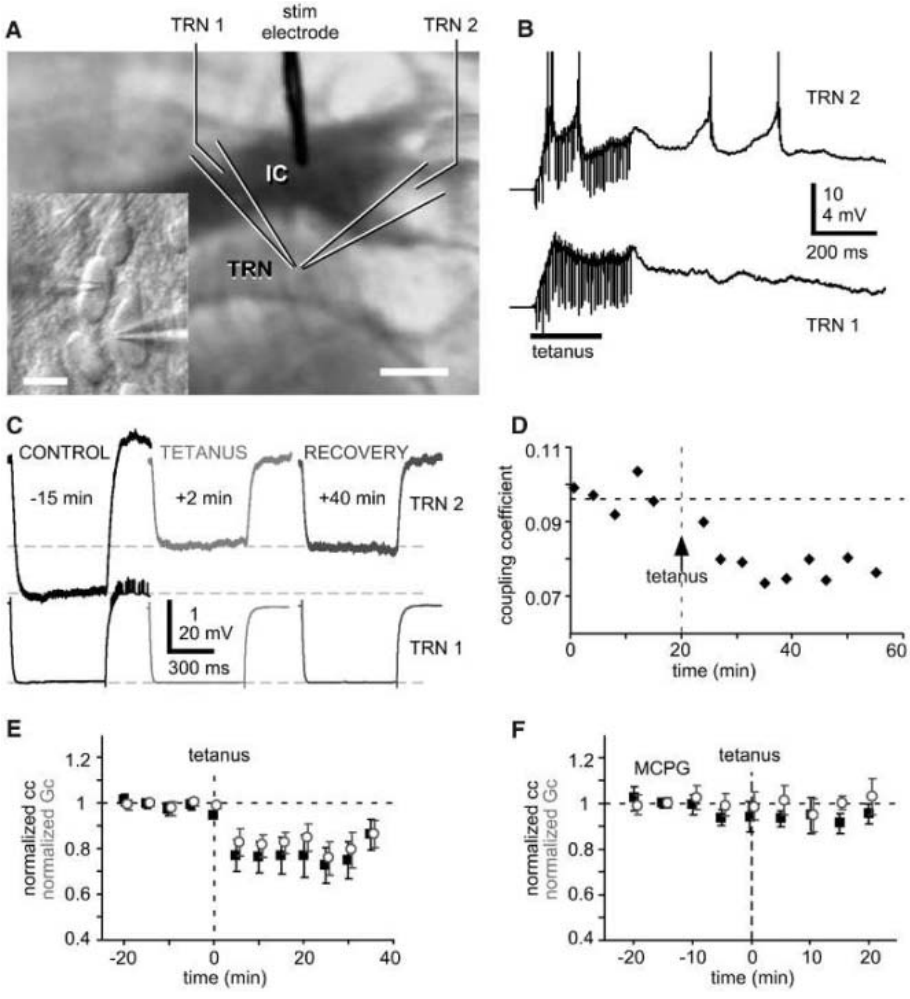

Fig. 1. Effects of tetanic stimulation on gap junction strength. (**A**) IR-DIC image of the thalamocortical slice with diagram of tetanus paradigm. Scale bars indicate 1 mm and 20 μm (inset). (Inset) High-magnification view of recorded neurons in TRN. (**B**) Responses of an electrically coupled neuron pair to tetanic stimulation. (**C**) Injected cell responses (TRN 1) and electrical coupling responses (TRN 2) before tetanus, after tetanus, and after recovery. Control cc equaled 0.11 and 0.07 immediately posttetanus to the end of recording. (**D**) Time-course measure of coupling coefficient in a pair of neurons before and after tetanus. (**E**) Normalized cc (black squares) and Gc (gray open circles) for nine pairs of cells before and after tetanic stimulation. *Y* values are normalized to the average of values collected before stimulation for each pair. (**F**) Normalized cc and Gc for four pairs before and after tetanic stimulation in the presence of MCPG. Time points represent 5-min bins, with 0 min equal to the time of tetanic stimulation. Each trace (C) and value per pair (D to F) is the average of 10 to 30 trials (mean ± SE).

We also tested the effects of modulating electrical synapses on electrical postsynaptic potentials (ePSPs) evoked by presynaptic spike bursts and by single spikes (burstlets and spikelets, respectively). ACPD consistently reduced the amplitude of both burstlets and spikelets (Fig. 3, A to D, and table S1) as well as their relative coupling strength (Fig. 3, E and F). The coupling strength of bursts and spikes were reduced by similar amounts [burst cc = –28.2 ± 7.1% and spike cc = –26.5 ± 9.9% (mean reduction ± SE), P = 0.58, two-tailed unpaired t test] (Fig. 3, E and F).

As described previously (*1*, *2*), electrical synapses between thalamic reticular neurons attenuate single spikes much more than bursts because of the low-pass filtering characteristics

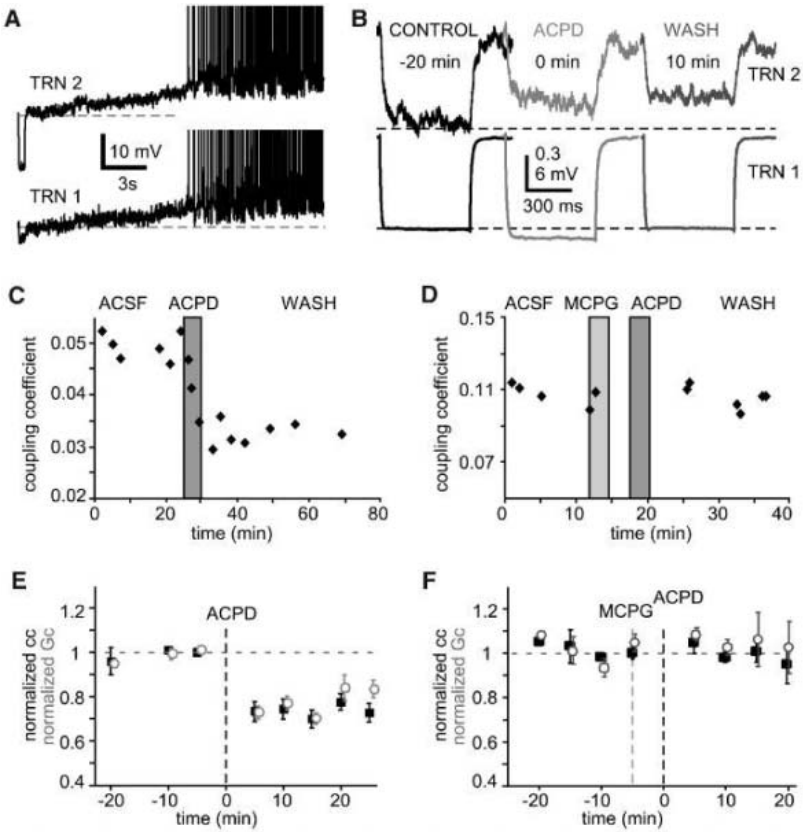

Fig. 2. Effects of ACPD on gap junction strength. (**A**) Cells depolarized to spiking during washing. A hyperpolarizing current step was delivered to TRN 1 (at trace start). (**B**) Step (–100 pA) responses (TRN 1) before, during, and after ACPD and corresponding gap junction responses (TRN 2). Control cc was 0.05 then 0.03 immediately post-tetanus through recovery. (**C**) cc time course of a neuron pair before and after ACPD application. (**D**) cc time course of a neuron pair before and after MCPG then ACPD application. Traces and time points show 10 to 30 trial averages (B to D). (**E**) cc and Gc time course for seven TRN pairs before and after ACPD application. (**F**) cc and Gc time course for five TRN pairs before drug application and after MCPG then ACPD wash. (E and F) *y* values normalized and *x* values binned as in Fig. 1F; each value per pair is the average of 10 to 30 trials. Gray bars (C and D) and dotted lines (E and F) indicate wash times. Error bars in (E) and (F) indicate SE.

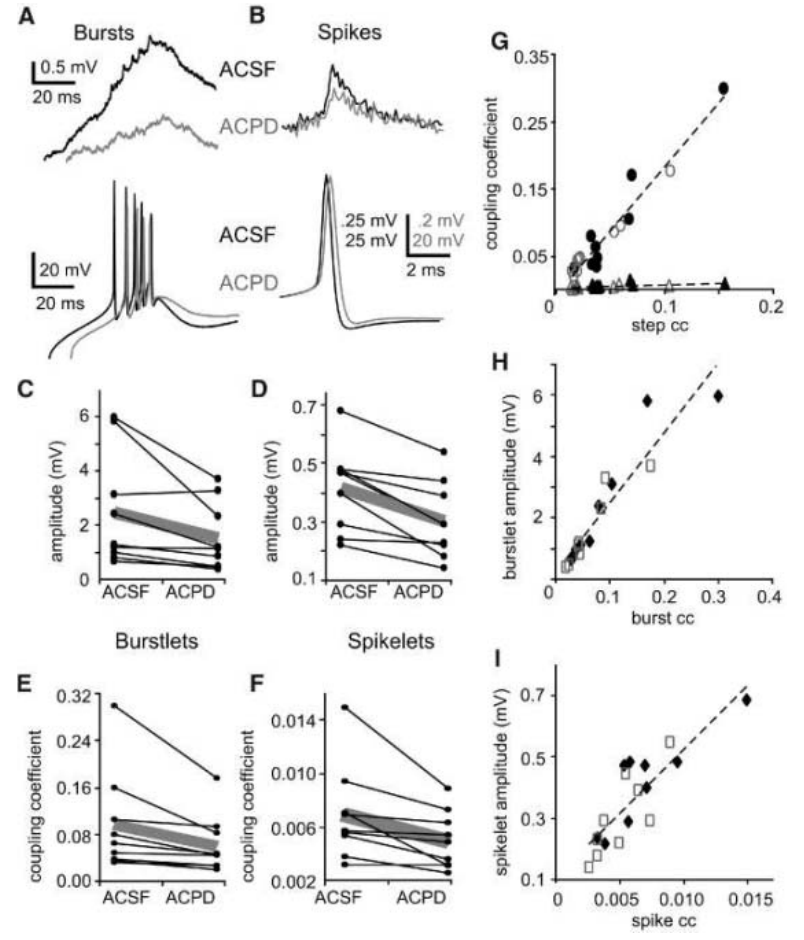

Fig. 3. Effect of ACPD modulation on gap junction transmission of spikes and bursts. (**A**) Control (black) and post-ACPD (gray) single bursts (bottom); postsynaptic burstlet responses directly above corresponding bursts. (**B**) Control (black) and post-ACPD (gray) single-action potentials (bottom) in ACSF (black) and post-ACPD (gray); spikelet responses above. (**C**) Paired average burstlet amplitudes before and after ACPD. (**D**) Paired average spikelet amplitudes before and after ACPD. (**E**) Paired average burst-to-burstlet cc before and after ACPD. Burst cc, pre- versus post-, $P < 0.04$. (**F**) Paired average spike-to-spikelet coupling coefficients before and after ACPD. Spike cc, pre- versus post-, $P < 0.03$. Two-tailed paired *t* tests (E and F). $N = 9$ cell pairs (C to F). Thick gray lines indicate population means (C to F). (**G**) The scaling relationship of direct current coupling coefficients versus burst and spike cc. Black solid symbols, control; gray open symbols, post-ACPD from the same neuron pairs; circles, burst cc; triangles, spike cc. Regression lines generated from ACSF and post-ACPD pooled data. Burstlet slope = 1.9 ($r^2 = 0.94$); spikelet slope = 0.05 ($r^2 = 0.41$). (**H** and **I**) Coupling strength versus amplitude of the postsynaptic event before and after ACPD. (H) Burstlets, slope = 23 ($r^2 = 0.87$). (I) Spikelets, slope = 42 ($r^2 = 0.73$). Burstlet data points are an average of two to four events per cell. Spikelet data points are the average of 10 to 20 events (C to I).

of the interconnected neurons and the slow voltage trajectory of a burst. On average, spike bursts evoke ePSPs with peak amplitudes about seven times larger than those of the ePSPs evoked by single spikes (table S1) [burstlet/spikelet amplitudes were 7.4 and 7.1 in artificial cerebro-spinal fluid (ACSF) and ACPD, respectively], even though the amplitudes of presynaptic spikes are about twice as large as the slow depolarizing envelope of bursts (table S1) (spike/burst amplitudes were 2.0 and 1.9 for ACSF and ACPD, respectively). The relative attenuation of bursts versus spikes is illustrated by the difference in the slopes (Fig. 3G) (*2*). Because ACPD caused very few persistent changes in the passive nonjunctional membrane properties of the neurons (table S1), there are no changes in these linear relationships of step attenuation versus burst and spike attenuation after ACPD. Additionally, the scaling factors for coupling strength versus amplitude of the postsynaptic events remained constant for burst and spike transmission (Fig. 3, H and I). The only persistent change that we observed was that most cells lost one spike per burst after ACPD application (n = 8 of 11 cells, range of 3 to 8 spikes per burst before ACPD and 2 to 7 after) (table S1).

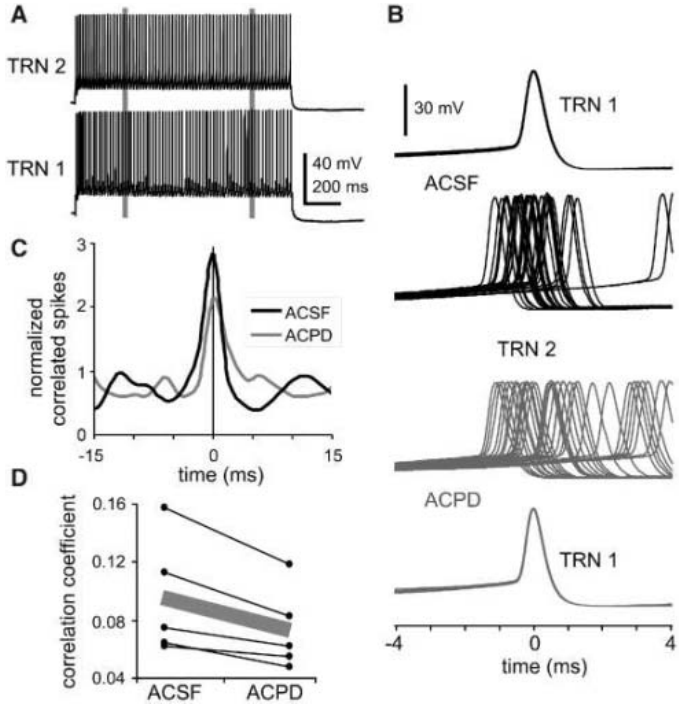

Fig. 4. Effect of reduced coupling strength on spike coordination. (**A**) Example responses of simultaneous spiking used to measure spike correlation before and after ACPD. Gray lines indicate correlation analysis window. (**B**) Demonstration of spike-triggered spikes in one trial before ACPD and one trial after for a TRN pair. The best single trial for each test was selected for comparison. Gray traces, post-ACPD, and black traces, controls (in ACSF). Top and bottom traces (TRN 1 trigger spikes) are the superimposed single spikes locked to their peaks (appear as one trace but have 40 spikes each). The middle traces (TRN 2 response spikes) are the spikes closest in time to each of the trigger spikes over an 8-ms window (±4 ms). (**C**) Cross-correlation of tonic action potentials before (black) and after (gray) ACPD for one TRN. *Y* axis normalized to average spikes per bin. Correlograms calculated from 10 consecutive trials each. (**D**) Paired correlation coefficients before and after ACPD (n = 5 cell pairs). Thick gray line is the population mean.

Presumably, one of the most important functions of gap junctions in the brain is to correlate the activity of coupled neurons (*10*–*13*). Electrical synapses of TRN cells can robustly synchronize the firing of neuron pairs under normal conditions (*1*, *2*). Thus, the reduction of coupling strength by mGluR activation could have important functional consequences for spike synchrony. We measured the effective coupling of pairs of neurons before and after ACPD and tested the strength of spike timing correlations during repetitive firing (Fig. 4). Weakening of synchrony is apparent in the single-trial example (Fig. 4B): Much larger jitter in spike timing of TRN2 relative to TRN1 is seen after ACPD treatment. Reduction of coordinated activity was also demonstrated by measuring cross-correlations of multiple trials (Fig. 4C). ACPD-induced reduction of electrical synapse strength was always accompanied by a decrease in spike synchrony, indicated by the correlation coefficients derived from cross-correlation analysis (Fig. 4D) (n = 5 pairs, P = 0.02, two-tailed paired t test).

mGluR activation (by endogenous glutamate or bath-applied agonist) caused a long-lasting, 20 to 30% reduction of electrical synapse strength between TRN neurons. The reduction of gap junction communication caused a consistent decrease in the size of both slow and fast postsynaptic events and weakened electrical synapse-mediated synchrony of spiking between pairs of TRN neurons.

Modulation of synapses by transmitters or activity has been studied extensively at mammalian chemical synapses. Modifiable strength expands the computational abilities of each synaptic contact. Activity-dependent modulation of neuronal electrical junctions, however, has not been previously characterized in the mammalian central nervous system (CNS).

Prior studies have shown gap junction modulation in other systems. Functional electrical synapses between TRN neurons require Cx36 (*1*, *11*, *13*). Cx35, the fish ortholog of Cx36, shares consensus phosphorylation sites with Cx36 (*14*). Cyclic adenosine monophosphate (cAMP) analogs cause a reduction in perch Cx35 hemichannel currents expressed in oocytes, presumably via a specific phosphorylation site (*14*). In synapses onto Mauthner cells, neural activity and activation of protein kinase II induce long-term changes of both chemical and electrical communication strength (*15*–*18*). The best evidence for modulation of Cx36 gap junctions comes from studies of retinal neurons (*19*). Mammalian amacrine cells have Cx36 gap junctions (*20*) that can be weakened by dopamine (*21*). There have also been suggestions of gap junctional modulation from tracer-coupling experiments done in neocortex (*22*), hypothalamus (*23*), and striatum (*24*) (although the relationship of these measurements to electrical coupling is unclear).

The gap junction modulation we describe here is functionally similar to mGluR-induced long-term depression at chemical synapses. In both cases, activation of mGluRs (by tetanus or agonist) induces slow postsynaptic effects lasting hundreds of milliseconds (Fig. 1B), and neuronal communication is then reduced by 20 to 50% for tens of minutes (*25*–*26*). Considering the similarities to chemical synapse modulation along with the bidirectional modulation seen at invertebrate electrical synapses, it is likely that electrical long-term potentiation (LTP) also exists at mammalian gap junction synapses (*15*–*17*).

The corticothalamic fibers originating from the deep layers of neocortex activate ionotropic AMPA, *N*-methyl-D-aspartate (NMDA), and mGluRs in cells of the mammalian dorsal thalamus (*4*–*7*). The excitatory action of the corticothalamic pathway plays a part in regulating the flow of information from sensory input, through relay nuclei of the thalamus, and into neocortex. Because mGluR activation reduces junctional coupling between TRN neurons, corticofugal input could effectively desynchronize inhibition of moderately coupled neurons that would otherwise be coordinated via electrical coupling. This decrease of coordinated inhibitory output from TRN could, in turn, enhance the efficacy of small amplitude excitatory events received by thalamic relay cells, making small sensory stimuli more salient during alert, exploratory states.

Another important role of TRN inhibition is to change the gating of thalamic relay cells from bursting to tonic mode, which is associated with changes from sleep to wakefulness (*27*, *28*). Reduced coupling strength between TRN cells during mGluR activation may contribute to the transition from thalamocortical

rhythms and burst firing to tonic firing of both TRN and relay cells (*29*). Thus, mGluRs may play a role in regulating the spatial and temporal coordination of inhibition to the dorsal thalamus.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5755/1809/DC1
Materials and Methods
Fig. S1
Table S1

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Glial Membranes at the Node of Ranvier Prevent Neurite Outgrowth

Jeffrey K. Huang,[1,2] Greg R. Phillips,[1] Alejandro D. Roth,[3] Liliana Pedraza,[3] Weisong Shan,[3] Wiam Belkaid,[3] Sha Mi,[4] Asa Fex-Svenningsen,[5] Laurence Florens,[6] John R. Yates III,[7] David R. Colman[1,3]*

Nodes of Ranvier are regularly placed, nonmyelinated axon segments along myelinated nerves. Here we show that nodal membranes isolated from the central nervous system (CNS) of mammals restricted neurite outgrowth of cultured neurons. Proteomic analysis of these membranes revealed several inhibitors of neurite outgrowth, including the oligodendrocyte myelin glycoprotein (OMgp). In rat spinal cord, OMgp was not localized to compact myelin, as previously thought, but to oligodendroglia-like cells, whose processes converge to form a ring that completely encircles the nodes. In OMgp-null mice, CNS nodes were abnormally wide and collateral sprouting was observed. Nodal ensheathment in the CNS may stabilize the node and prevent axonal sprouting.

Myelin sheaths that wrap around neuronal axons are periodically interrupted by nodes of Ranvier that enable saltatory conduction (*1*) (Fig. 1A). After peripheral nervous system (PNS) injury, axonal sprouting from neighboring unlesioned nerves commonly occurs at nodes of Ranvier, allowing for the reestablishment of functional neurocircuitry (*2*, *3*). In contrast, injury-induced sprouting rarely occurs at CNS nodes despite the absence of myelin, which is inhibitory to neurite outgrowth (*4*). One explanation for the nonresponsiveness of CNS axons to injury might be that sprouting is prevented by nonmyelin-derived factors present in the nodal vicinity.

To identify such inhibitory factors, we isolated membranes of the mammalian CNS nodal axoglial apparatus, comprising the node and flanking paranodal domains, by subcellular fractionation of dissected bovine, mouse, or human white matter. Because the nodal axoglial apparatus is morphologically distinct from myelin (Fig. 1B) and likely to be of greater density due to a higher protein:lipid ratio than myelin, we reasoned that membranes of the nodal axoglial apparatus might be sheared away during homogenization and be concentrated at an isopycnic density greater than that of compact myelin. We modified a synaptosome protocol (*5*) by using as starting material CNS white matter that contained myelinated axons and was devoid of synaptic endings. Membrane fractions were recovered from sucrose density gradients at a 0.32/1.0 M interface, comprising compact myelin membranes (*6*), and at a 1.0/1.25 M interface, which we provisionally termed "axogliasomes." Ultrastructural examination of axogliasomes revealed membrane profiles characteristic of paranodal loops, attached to underlying axolemmal fragments (Fig. 1C). In contrast, profiles of compact myelin and synaptosomes were rarely detected. Immunoelectron microscopy examination with a paranode-specific marker, Caspr (*7*, *8*), confirmed that the observed membranes were derived from the nodal axoglial apparatus (Fig. 1D). We also investigated whether axogliasomes contained appropriate biochemical markers for the nodal axoglial apparatus. The known paranodal markers Caspr, contactin, and neurofascin-155 (*9*), as well as the nodal marker, neurofascin-186 (*10*), were all detected by Western blot analysis of the axogliasome fraction, whereas compact myelin-specific markers, the proteolipid proteins PLP and DM20, were barely detected (Fig. 1E). The detection of myelin-associated glycoprotein (MAG) in compact myelin and axogliasomes was expected, because it is expressed throughout periaxolemmal channels of compact myelin and at paranodes (*11*). Axogliasomes are thus morphologically and biochemically distinct from membranes derived from compact myelin and comprise the entire nodal axoglial apparatus.

It is well established that purified compact myelin membranes can effectively limit neurite outgrowth activity in cell culture owing to myelin-specific inhibitory factors (*4*). To determine if CNS nodes of Ranvier also contain inhibitory factors for axon outgrowth,

[1]Fishberg Department of Neuroscience, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, USA. [2]Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, UK. [3]The Montreal Neurological Institute, McGill University, 3801 University Street, Montreal PQ H3A 2B4, Canada. [4]Biogen Idec, Discovery Biology, 14 Cambridge Center, Cambridge, MA 02142, USA. [5]Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, SE-751 85 Uppsala, Sweden. [6]Stowers Institute, 1000 East 50th Street, Kansas City, MO 64110, USA. [7]Department of Cell Biology SR11, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

*To whom correspondence should be addressed. E-mail: david.colman@mcgill.ca

we seeded embryonic rat cerebellar (Cb) neurons onto axogliasome-spotted culture chambers at increasing protein concentrations, and axon lengths were measured after 36 hours in culture. For comparison, we also seeded Cb neurons onto compact myelin-spotted chambers. The outgrowth capacity of Cb neurons was limited in the presence of axogliasomes, and sprouting decreased exponentially as the concentration of axogliasome proteins increased from as low as 50 ng/μl (Fig. 1F). We found that Cb neurites were on average 20 nm in length at 100 ng/μl axogliasome concentration—a near-fourfold decrease compared with control water-spotted cultures (Fig. 1H). We also found that neurite lengths decreased by nearly eightfold compared with water-spotted cultures as the concentration of axogliasomes increased up to 1000 ng/μl. Indeed, the inhibitory effect of axogliasomes on axon outgrowth was comparable to that of myelin-coated culture chambers at the same protein concentrations tested (Fig. 1, F and H). To determine if the nonpermissive effect of axogliasomes also applied to postnatal neurons, we dissected dorsal root ganglia (DRG) from postnatal day 8 (P8) rats, placed the ganglia directly onto axogliasome-coated coverslips at increasing protein concentrations, and incubated them for 7 days in culture before measuring their axon lengths. We observed the outgrowth capacity of DRG axons reduced by about fourfold, from greater than 4 mm to less than 1 mm in length, when axogliasomes concentrations increased from 25 to 1000 ng/μl (Fig. 1, G and I). These observations suggest the presence of potent inhibitory peptides at the CNS node of Ranvier that limit axon outgrowth activity in both embryonic and postnatal neurons.

To identify the protein constituents that might mediate neurite outgrowth activity, we subjected axogliasomes to proteomic analysis by multidimensional protein identification technology (MudPIT) (*12*). MudPIT detects peptides comprising all isoelectric points, molecular weights, and hydrophobicities, including integral membrane and low-abundance proteins. Rather than analyzing peptides derived from excised gel pieces, it separates and further fragments peptides from a solubilized membrane fraction by a combination of multidimensional liquid chromatography and tandem mass spectrometry (MS/MS), followed by an automated sequence analysis of individual peptide fragments eluted from the chromatography column. More than 300 nonredundant proteins were identified from axogliasomes (Fig. 2A), including many with known localization to the nodal axoglial apparatus (Fig. 2B). We also identified proteins that were previously unsuspected as constituents of the nodal axoglial apparatus (Fig. 2B), such as a disintegrin and metalloprotease protein 23 (ADAM23) (*13*), and collapsin response mediator protein 2 (Crmp-2). Several known negative regulators of neurite outgrowth (Fig. 2B) were also identified, including MAG, versican, protein kinase C (*14*–*17*), and the oligodendrocyte myelin glycoprotein (OMgp) (*18*, *19*). By Western blot analysis, OMgp was detected at the expected molecular sizes of 95 to 120 kD (*20*) and was present in both CNS white matter membranes and axogliasomes (Fig. 2C).

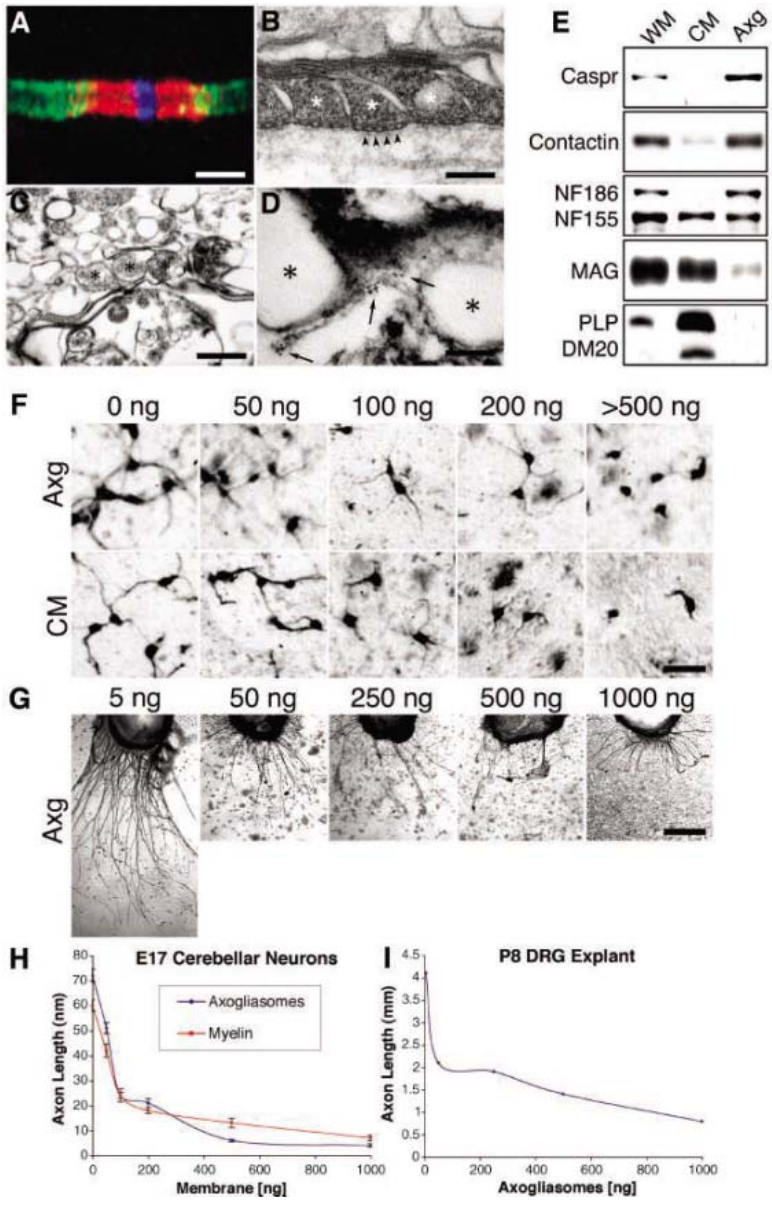

Fig. 1. Isolated membranes of CNS nodal axoglial apparatus prevent neurite outgrowth. (**A**) Longitudinal section of P15 rat spinal cord labeled with antibody to sodium channel (anti–sodium channel) (blue) at the node of Ranvier, anti-Caspr (red) at the paranode, and anti–potassium channel (green) at the juxtaparanode. Bar, 2.5 μm. (**B**) Longitudinal section of rat CNS revealed the paranode, comprising characteristic paranodal loops (asterisks) attached to the axolemma through septate densities (arrowheads). Bar, 180 nm. (**C**) Bovine axogliasomes contained 250- to 500-nm vesicles (asterisks) attached to one another and to underlying axolemmal fragments. Bar, 550 nm. (**D**) Detection of Caspr-gold particles (arrows) in isolated paranodal membranes (asterisks). Bar, 375 nm. (**E**) Western blot analysis demonstrating the enrichment of proteins from the nodal axoglial apparatus in axogliasomes (Axg) isolated from bovine white matter (WM) compared with compact myelin (CM). (**F**) At increasing mouse axogliasome (Axg) or compact myelin (CM) protein concentration, embryonic day 17 rat Cb neurite outgrowth decreases. Bar, 50 nm. (**G**) P8 rat DRG explants were cultured on axogliasome at the indicated protein concentrations. Bar, 1 mm. (**H**) Measurement of E17 rat Cb neurite outgrowth lengths under increasing Axg and CM concentrations (>50 neurites/well). Values are means ± SEM. (**I**) Measurements of mean outgrowth lengths of P8 rat DRG axons under increasing concentration of axogliasomes.

Although its precise distribution had not been demonstrated, OMgp has generally been regarded as a component of myelin membranes (*20*). OMgp was not detected in compact my-

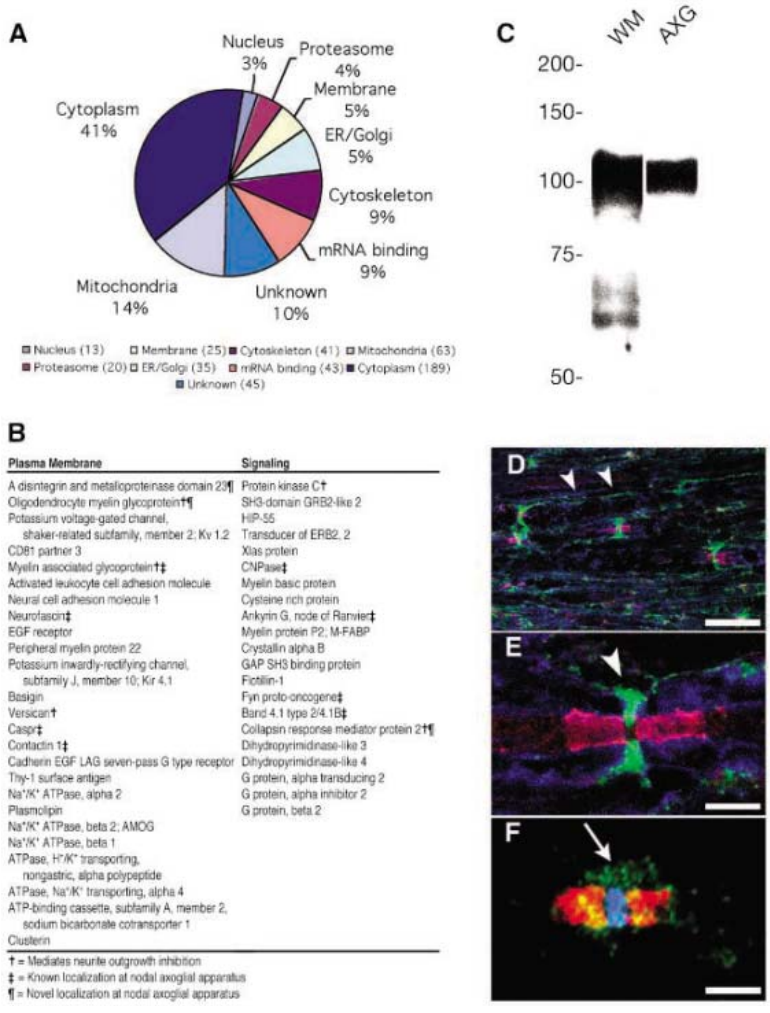

Fig. 2. Proteomic dissection of human axogliasomes reveals OMgp as a component of CNS nodal axoglial apparatus. (**A**) Functional profile of MudPIT-identified proteins. (**B**) List of candidate proteins identified from axogliasomes. (**C**) Western blot detection of OMgp at 95 to 120 kD in human white matter (WM) and axogliasomes (AXG). The lower molecular size bands detected in WM might represent degraded protein fragments, because they appear not to be present in AXG. (**D**) Longitudinally sectioned P18 rat spinal cord labeled with anti-OMgp (green) demonstrates nodal distribution. Arrowheads indicate glial processes extending on the surface of myelin before terminating at nodes of Ranvier. Paranodes were labeled with anti-Caspr (red) and compact myelin was labeled with Rip (blue). Bar, 10 μm. (**E**) At higher magnification in a sectioned P40 rat spinal cord, OMgp (green) is detected in a prominent membranous ensheathment (arrowhead) that encircles the axon at the node of Ranvier. Bar, 2 μm. (**F**) In P18 teased rat sciatic nerves, OMgp (green) was observed in PNS nerves at Schwann cell microvilli that encircle the node (arrow). Paranodes were labeled with anti-Caspr (red) and node with anti–sodium channel (blue). Bar, 2 μm.

elin in the CNS, but rather was consistently detected in thin glial membranes that appear to extend on the outer surface of myelin and terminate at nodes of Ranvier (Fig. 2D). Notably, OMgp was localized to a sharply defined, tight membranous "ring" encircling the CNS axon at the node of Ranvier (Fig. 2E). Its weak expression at the outer myelin surface resembled that of MOG, a protein component of noncompact myelin expressed in the outermost membrane (*21*) but not at the nodal locus, as we found for OMgp. OMgp was also detected at nodes of Ranvier in PNS fibers (Fig. 2F), albeit at a much lower relative labeling intensity, and in a diffuse circumnodal pattern. Its position at PNS nodes suggests that OMgp might be a constituent of Schwann cell microvilli, which are membranous extensions of Schwann cell myelin that engage each other across the node of Ranvier. However, CNS nodes do not exhibit microvillous ensheathment and appear bare ultrastructurally (*22*); this suggested that OMgp might be expressed at CNS nodes by a different glial cell type. In transverse serial sections of a CNS nodal axoglial apparatus, we detected multiple glial processes expressing OMgp (OMgp$^+$) that converged on and completely ensheathed the axon at the node of Ranvier (Fig. 3A, movie S1). In cerebellar cultures, we also observed OMgp$^+$ processes in contact with developing nodes of Ranvier that flanked the elongating myelin membranes (Fig. 3C). These results suggest that OMgp is derived from a nonmyelinating glial cell type and directly interacts with a nodal component on the axon.

Previous studies have suggested the existence of astrocyte-like cells that contact the node of Ranvier (*23*, *24*). However, no convincing molecular markers for this cell type were described, and it remained unclear if these contacts were passive, or whether they were features of all CNS nodes. OMgp was not detected in conventional astrocytes, because its expression did not codistribute with the astrocyte-specific marker GFAP (glial fibrillary acidic protein) (Fig. 3F). OMgp$^+$ cells exhibited a stellate morphology (Fig. 3, D and E) that strongly resembled that of NG2 proteoglycan-expressing (NG2$^+$) oligodendrocyte precursor cells (OPCs) (*25*) (Fig. 3, G and H). NG2$^+$ cells are abundantly expressed in the adult CNS, and NG2 proteoglycans have also been shown to inhibit neurite outgrowth in vitro (*25*, *26*). Like OMgp$^+$ cells, NG2$^+$ glial processes extended toward and encircled CNS nodes of Ranvier in the spinal cord (Fig. 3B, movie S2). It was unclear how many nodes were ensheathed by NG2$^+$ processes because NG2 staining is most prominently detected in glial cell bodies and their processes, whereas OMgp is most prominently detected at the terminals of glial cell processes that surround the nodes of Ranvier. Although we were not able to directly demonstrate colocalization of NG2 and OMgp within the same cell with the available antisera to each protein, the observation of identical cellular morphology and the tight nodal ensheathment by NG2$^+$ and OMgp$^+$ glial processes, particularly in transverse spinal cord sections (Fig. 3, E and H), suggested that this is the same cell type and might represent a mature population of nonmyelinating oligodendrocytes. These cells might be similar to the recently described adult NG2$^+$ glia that contact CNS nodes in the rat optic nerve (*27*).

The detection of OMgp$^+$ glial processes at the node of Ranvier implies that they might participate in nodal formation and maintenance. Furthermore, the inhibitory nature of the ensheathing processes suggests that nodal ensheathment by OMgp$^+$ cells might be necessary to prevent collateral axon outgrowth during development. To address these issues, we generated a mutant mouse lacking OMgp (OMgp$^{-/-}$) by deletion of the entire coding sequence of the OMgp gene (*28*). OMgp$^{-/-}$ mice did not exhibit obvious behavioral abnormalities compared with the wild type at all ages examined (from P0 to P30). There also did not appear to be any changes in NG2$^+$ cell numbers in the mutant mouse spinal cord. However, in spinal cord sections of P21 mutant mice, the axonal marker, Caspr, which sharply flanks the node in wild-type animals (Fig. 4B), was observed either in a diffuse pattern or ec-

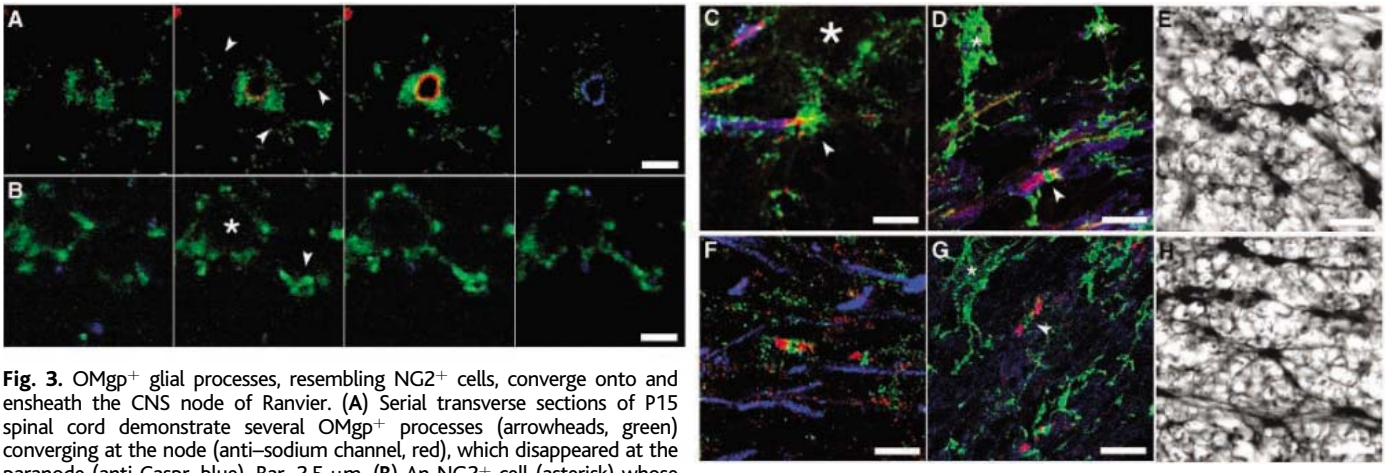

Fig. 3. OMgp$^+$ glial processes, resembling NG2$^+$ cells, converge onto and ensheath the CNS node of Ranvier. (**A**) Serial transverse sections of P15 spinal cord demonstrate several OMgp$^+$ processes (arrowheads, green) converging at the node (anti–sodium channel, red), which disappeared at the paranode (anti-Caspr, blue). Bar, 2.5 μm. (**B**) An NG2$^+$ cell (asterisk) whose process (arrowhead, green) is observed to encircle the node. Bar, 5 μm. (**C**) Immunostaining of Cb cultures demonstrates an OMgp-expressed cell (asterisk) whose process (green, arrowhead) contacts a developing heminode flanked by a paranode (anti-Caspr, red) and myelinating membranes (anti–myelin basic protein, blue). Bar, 7.5 μm. (**D**) P15 rat spinal cord revealed OMgp$^+$ glial cells (asterisks, green) resembling oligodendrocyte precursor cells. Arrowhead points to a node. Bar, 7.5 μm. (**E**) Immunoperoxidase labeling of P18 spinal cord transverse section demonstrates abundant OMgp$^+$ cells with stellate morphology. Bar, 15 μm. (**F**) GFAP (blue) expressed by astrocytes do not colocalize with OMgp (green). Bar, 5 μm. (**G**) An oligodendrocyte precursor-like cell (asterisk, green) labeled with anti-NG2 proteoglycan. NG2$^+$ processes were observed to contact the node (arrowhead). Bar, 8 μm. (**H**) NG2$^+$ cells resemble the OMgp$^+$ stellate cells. Bar, 14 μm.

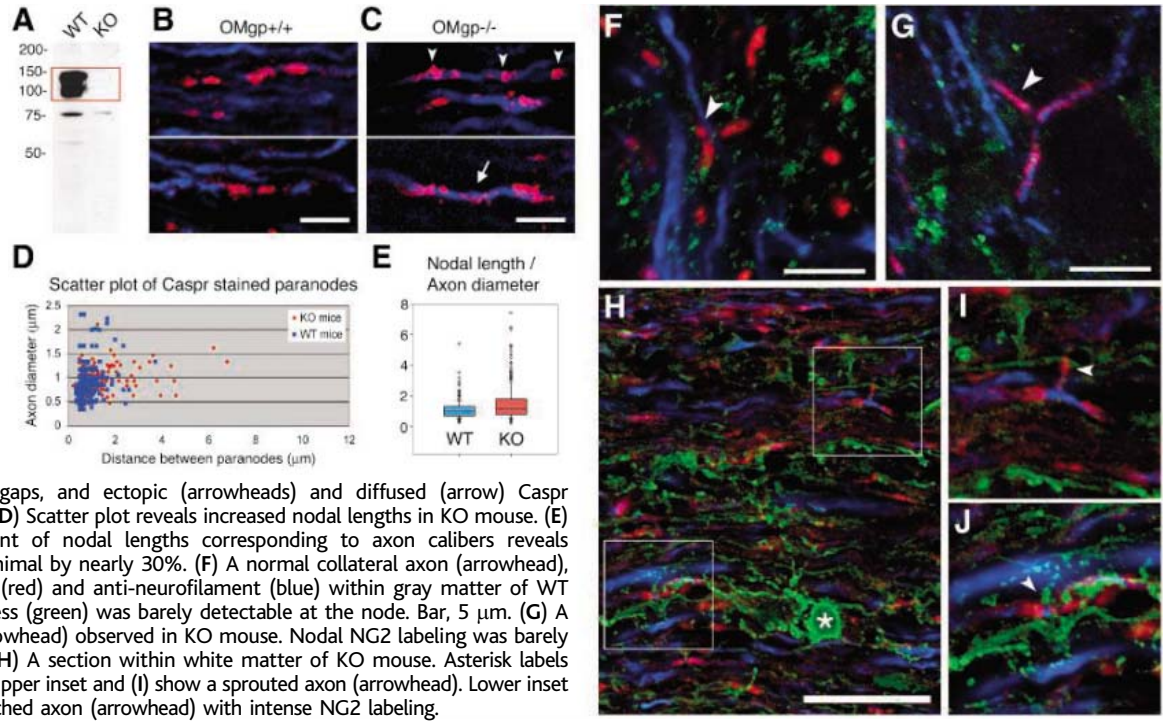

Fig. 4. OMgp-null mouse exhibits abnormal CNS nodes and collateral sprouting. (**A**) OMgp proteins are absent in spinal cord extract of OMgp-null (KO) mouse compared with the wild type (WT). Red box indicates OMgp. (**B**) P21 WT spinal cord (OMgp+/+) shows narrow nodal gaps between adjacent paranodes (anti-Caspr, red). Axons were labeled with anti-neurofilament (blue). Bar, 5 μm. (**C**) KO spinal cord (OMgp−/−) shows widened nodal gaps, and ectopic (arrowheads) and diffused (arrow) Caspr distribution. Bar, 5 μm. (**D**) Scatter plot reveals increased nodal lengths in KO mouse. (**E**) Normalized measurement of nodal lengths corresponding to axon calibers reveals widened nodes in KO animal by nearly 30%. (**F**) A normal collateral axon (arrowhead), labeled with anti-Caspr (red) and anti-neurofilament (blue) within gray matter of WT spinal cord. NG2$^+$ process (green) was barely detectable at the node. Bar, 5 μm. (**G**) A thin collateral axon (arrowhead) observed in KO mouse. Nodal NG2 labeling was barely detectable. Bar, 5 μm. (**H**) A section within white matter of KO mouse. Asterisk labels NG2$^+$ cell. Bar, 24 μm. Upper inset and (**I**) show a sprouted axon (arrowhead). Lower inset and (**J**) show an unbranched axon (arrowhead) with intense NG2 labeling.

topically clustered on the axon (Fig. 4C). Indeed, nodal lengths, which are ~0.5 to 1 μm in normal mice, were much more variable in mutant mice, ranging from ~0.5 to 7 μm (Fig. 4D). Because nodal sizes may vary owing to differences in axon caliber, we calculated the ratio of measured nodal lengths to measured axon diameters and found that on average, nodal length was ~30% greater in the mutant than in wild-type animals (Fig. 4E). These observations indicate that the establishment of CNS nodes is impaired in OMgp-null mice and that OMgp is likely required for this process to proceed at a normal pace.

Several discrete axons sprouting from nodes of Ranvier were observed in OMgp-null mice in both gray (Fig. 4G) and white matter regions (Fig. 4, H and I). At least 30 nodal sprouts were detected in 10 mutant mouse spinal cord sections. These bifurcated axons exhibited wider nodal gaps and abnormal Caspr labeling, suggesting that the loss of OMgp might enable collateral sprouting. We also observed, but with lower frequency, occasional collateral sprouting in gray and white matter regions of wild-type spinal cord, in which we detected 12 sprouts from 10 examined spinal cord sections (Fig. 4F). These are likely normally branched axons; in these cases, nodal gaps and Caspr staining appeared no different from unbranched axons. It is extremely unlikely that the observed sprouted axons in OMgp-null mice were the result of nearby axons crossing at the node, because we inspected each of the individually collected confocal laser scanned slices (at 0.1 μm per slice) from

all imaged spinal cord sections to rule out possible axon intersections. Notably, in all of the collaterally sprouted axons from both mutant and wild-type animals, NG2$^+$ glial processes were barely detectable at the nodal vicinity (Fig. 4I), whereas many nonsprouted nodes appeared to be in contact with NG2$^+$ glial processes (Fig. 4J). This observation further indicates that nodal ensheathment is necessary to prevent axonal sprouting.

In conclusion, we find that isolated membranes of CNS nodes of Ranvier are nonpermissive for neurite outgrowth and propose that this is due to cell extensions that emanate from NG2$^+$ oligodendrocyte precursor-like cells that tightly ensheath the nodal axon. It is known that NG2 is expressed by a heterogeneous population of neuroglial cells in the adult CNS, ranging from oligodendrocyte precursors to specialized glial cells that contact nodes of Ranvier in optic nerves and synaptic terminals in the hippocampus (*29*–*31*). Whether these nodal glial cells represent the same lineage of specialized NG2$^+$ neuroglia described to contact nodes of Ranvier in rat optic nerves remains to be determined. Furthermore, we found glial processes emanating from specialized oligodendrocyte-like cells that converge at the CNS node and contain high concentrations of the neurite outgrowth inhibitor, OMgp. OMgp was detected at most of the nodes examined, which suggests that it may function in generating normal nodal architecture and in suppressing collateral sprouting, because in OMgp-null mice, the nodal gap is abnormally widened and sprouting from this locus is observed. It also remains to be determined whether OMgp is necessary for survival of the NG2$^+$ oligodendrocyte precursor-like cells, although given the lack of decrease in NG2$^+$ cells in the OMgp$^{-/-}$ mice, this may not be a likely functional role for OMgp. It seems more likely that OMgp plays a role in the adhesion of NG2$^+$ glial processes to CNS nodes because in all of the observed sprouted nodes, there were no detectable NG2$^+$ processes.

Finally, considering the intimate relation between the node and the encircling inhibitory glial membranes, after traumatic injury to the CNS, OMgp and possibly other inhibitory peptides at the nodal/paranodal region, such as versican, MAG, NG2, or Nogo-A (*11*, *16*, *27*, *32*), may remain stably attached to the nodal region or become deposited on the axonal surface, thus preventing axons from responding to injury-elicited growth signals. Overcoming the inhibitory nature of these nodal glial cells may yield new therapeutic interventions to promote functional recovery after CNS trauma.

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The Widespread Impact of Mammalian MicroRNAs on mRNA Repression and Evolution

Kyle Kai-How Farh,[1]* Andrew Grimson,[1]* Calvin Jan,[1] Benjamin P. Lewis,[1,2] Wendy K. Johnston,[1] Lee P. Lim,[3] Christopher B. Burge,[2] David P. Bartel[1]†

Thousands of mammalian messenger RNAs are under selective pressure to maintain 7-nucleotide sites matching microRNAs (miRNAs). We found that these conserved targets are often highly expressed at developmental stages before miRNA expression and that their levels tend to fall as the miRNA that targets them begins to accumulate. Nonconserved sites, which outnumber the conserved sites 10 to 1, also mediate repression. As a consequence, genes preferentially expressed at the same time and place as a miRNA have evolved to selectively avoid sites matching the miRNA. This phenomenon of selective avoidance extends to thousands of genes and enables spatial and temporal specificities of miRNAs to be revealed by finding tissues and developmental stages in which messages with corresponding sites are expressed at lower levels.

MicroRNAs are an abundant class of endogenous ~22-nucleotide (nt) RNAs that specify posttranscriptional gene repression by base-pairing to the messages of protein-coding genes (*1*, *2*). Hundreds of miRNAs have been identified in humans (*1*), and thousands of messages are under selection to maintain pairing to miRNA seeds (nucleotides 2 to 7 of the miRNA), enabling regulatory targets of miRNAs to be predicted simply by searching 3′ untranslated regions (3′UTRs) for evolutionarily conserved 7-nt matches to miRNA seed regions (*3*–*5*).

We used the mouse expression atlas (*6*) to examine the expression of the predicted targets of six tissue-specific miRNAs: miR-1 and miR-133 (skeletal muscle), miR-9 and

[1]Whitehead Institute for Biomedical Research, Department of Biology, Massachusetts Institute of Technology, and Howard Hughes Medical Institute, 9 Cambridge Center, Cambridge, MA 02142, USA. [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. [3]Rosetta Inpharmatics, 401 Terry Avenue North, Seattle, WA 98109, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: dbartel@wi.mit.edu

miR-124 (brain), miR-122 (liver), and miR-142-3p (hematopoietic organs and blood cells) (*7*–*10*) (fig. S1). The 250 messages with conserved miR-133 sites were generally expressed in muscle but at lower levels in muscle than in other tissues (Fig. 1A). Similarly, predicted targets of the other miRNAs were usually at lower levels in the tissue expressing the miRNA than in other tissues (Fig. 1A). Brain-specific miR-9 and miR-124 displayed more complex patterns, perhaps reflecting the heterogeneous cell types within the brain.

The low relative expression of predicted targets in differentiated tissues raised the question of whether they might be more highly expressed earlier in differentiation, before miRNA expression. To address this, we analyzed expression profiles of myotube differentiation (*11*), during which miR-1 and miR-133 accumulate after cell-cycle arrest (*12*). Predicted targets of these muscle-specific miRNAs were preferentially high before miRNA expression and then dropped as the miRNAs accumulated (Fig. 1B and fig. S3). Our observation that miRNAs induced during differentiation tend to target messages highly expressed in the previous developmental stage suggested a function analogous to that proposed for miRNAs in plants: They dampen the output of preexisting messages to facilitate a more rapid and robust transition to a new expression program (*13*). Predicted targets tended to be expressed at substantial levels on the absolute scale (Fig. 1A, *x* axis), which further suggested that metazoan miRNAs are often optimizing protein output without eliminating it entirely (*14*).

Our results are consistent with the idea that miRNAs are destabilizing many target messages to further define tissue-specific transcript profiles (*15*) but also leave open the possibility that many targets are repressed translationally without mRNA destabilization. If miRNAs were usually working in concert with transcriptional and other regulatory processes to down-regulate the same genes, then a correlation between conserved targeting and lower mRNA levels would be observed even for messages that miRNAs translationally repress without destabilizing.

Mammalian miRNA families have an average of ~200 conserved targets above estimated background, a figure approximately 1/10th the number of 3′UTRs with 7-nt sites in a single genome (*3*, *5*). Computational algorithms rely on evolutionary conservation to distinguish functional miRNA targets from the thousands of messages that would pair equally well; in contrast, the cell must rely on specificity determinants intrinsic to a single genome. To determine whether these nonconserved sites might be functional, we used reporter assays to compare repression mediated by conserved and nonconserved sites. We selected two targets of miR-1, predicted by TargetScan based on conservation in human, mouse, and rat (*16*) and six human UTRs that had comparable TargetScan scores in human but low or nonexistent scores in mouse or rat. When eight UTR fragments of ~0.5 kb that contained the sites were placed in reporters, we observed specific repression for all of them (Fig. 2A). Analogous experiments with eight predictions from our more sensitive analysis, TargetScanS, which searches for conserved 7- or 8-nt matches (*3*), and 17 genes with nonconserved matches also detected little difference between UTR fragments containing conserved and nonconserved sites (Fig. 2B), even when the concentration of transfected miRNA was titrated to suboptimal levels (fig. S4). Apparently, most nonconserved sites fortuitously reside in local contexts suitable for mediating repression and therefore have the potential to function when exposed to the miRNA. These results generalize previous work showing that in certain contexts 7- or 8-nt matches appear sufficient for miRNA-like regulation (*4*, *17*, *18*). We conclude that additional recognition features, such as pairing to the remainder of the miRNA, accessible mRNA structure, or protein-binding sites, are usually dispensable, or occur so frequently that they impart little overall specificity [supporting online material (SOM) text].

To explore the impact of this vast potential for nonconserved targeting, we examined the expression of messages with nonconserved 7-nt matches to tissue-specific miRNAs, focusing first on messages with sites present in mouse but not in the orthologous human UTRs (Fig. 3A). In contrast to the conserved sites, the nonconserved sites had a propensity to fall in the UTRs of genes that were not expressed in the same tissue as the miRNA. Also notable was the depletion of sites among those genes that were most highly and specifically expressed in the tissue. Such depletion could result primarily from direct miRNA-mediated destabilization of messages (*15*), or some depletion might be from selective avoidance of sites—evolutionary pressure for messages highly specific to a tissue to lose sites for coexpressed miRNAs.

To distinguish between these two possibilities, we plotted the expression, in mouse,

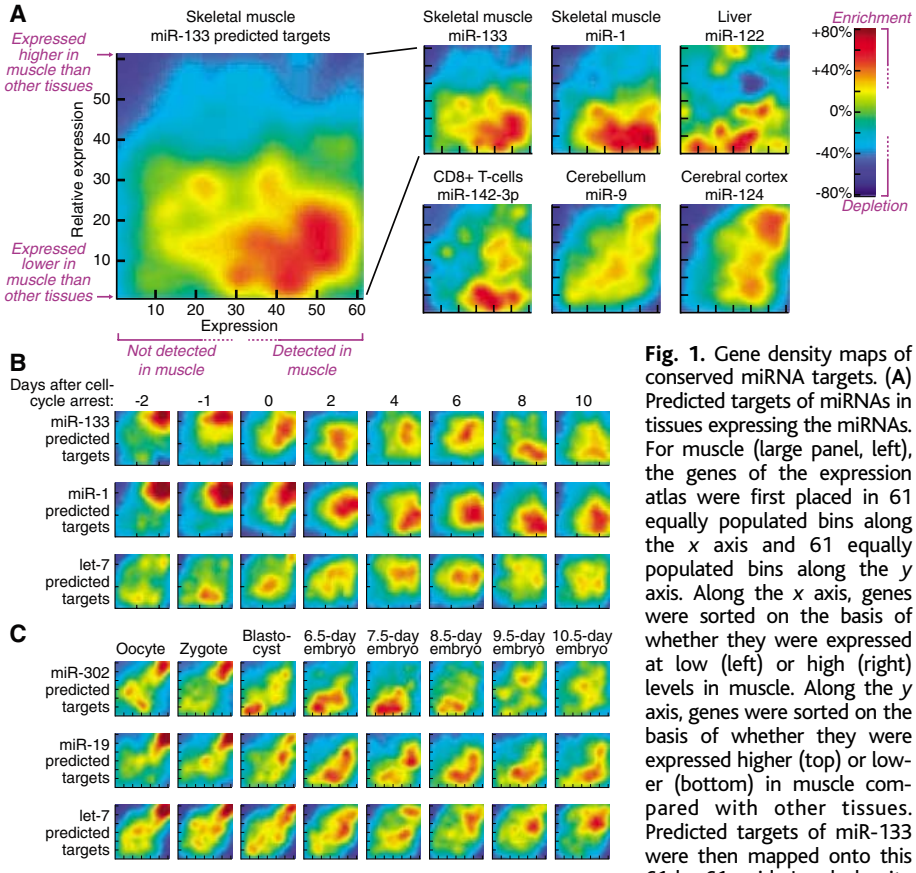

Fig. 1. Gene density maps of conserved miRNA targets. (**A**) Predicted targets of miRNAs in tissues expressing the miRNAs. For muscle (large panel, left), the genes of the expression atlas were first placed in 61 equally populated bins along the *x* axis and 61 equally populated bins along the *y* axis. Along the *x* axis, genes were sorted on the basis of whether they were expressed at low (left) or high (right) levels in muscle. Along the *y* axis, genes were sorted on the basis of whether they were expressed higher (top) or lower (bottom) in muscle compared with other tissues. Predicted targets of miR-133 were then mapped onto this 61-by-61 grid. Local density [after background subtraction (fig. S2) and smoothing] of miR-133 targets is color coded, with regions of enrichment (red) or depletion (blue) shown (key at far right). Other miRNA-tissue pairs were analyzed analogously (smaller panels, right). (**B**) Time course of predicted targets during myoblast (C2C12) differentiation to myotubes, analyzed with a 24-by-24 grid. (**C**) Time course of predicted targets during mouse embryogenesis, analyzed as in (A). Predicted targets of let-7 are included for comparison in (B) and (C).

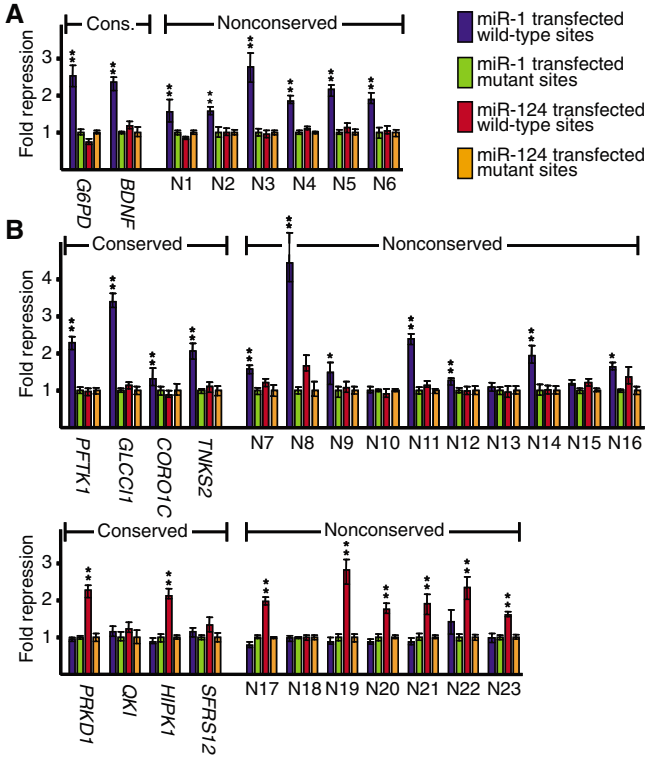

Fig. 2. MicroRNA-mediated repression of luciferase reporter genes containing 3′UTR fragments with conserved or nonconserved sites. (**A**) UTR fragments with TargetScan-like miR-1 sites. Luciferase activity from HeLa cells cotransfected with miRNA and wild-type reporters was normalized to that from cotransfection with mutant reporters with three point substitutions disrupting each seed match. The miR-124 transfections served as specificity controls. Error bars represent the third largest and smallest values among 12 replicates (one asterisk, $P < 0.01$; two asterisks, $P < 0.001$, Wilcoxon rank-sum test). (**B**) UTR fragments with TargetscanS-like miR-1 (top) and miR-124 (bottom) sites, analyzed as in (A).

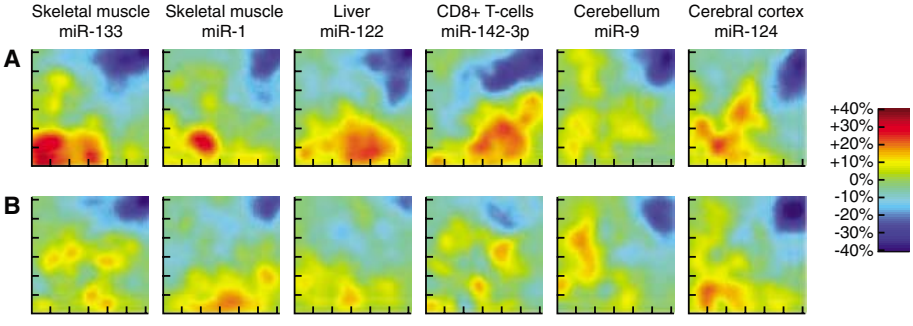

Fig. 3. Density maps for genes with nonconserved sites. (**A**) Messages with site present in mouse 3′UTR but absent in human ortholog. Data are shown as in Fig. 1, but enrichment is relative to matched cohorts (figs. S5 and S6), controlling for UTR length and nucleotide composition. (**B**) Messages with site present in human UTR but absent in orthologous mouse UTR, analyzed as in (A).

of genes that lacked sites in the mouse UTR but contained a site in the human ortholog. Because such messages would not be subject to miRNA-mediated destabilization in mouse, the depletion signal would vanish if it reflected only direct destabilization. However, the signal persisted (Fig. 3B, blue in upper right); mouse genes that were highly and specifically expressed in the tissue were less likely to harbor sites in their human orthologs, indicating that genes preferentially coexpressed with the miRNA have evolved to avoid targeting by that miRNA. The enrichment for genes expressed at low levels also explained some of the many potentially functional nonconserved sites; they accumulate by chance, without consequence, in messages not coexpressed with the miRNA. The reduction in signal in Fig. 3B compared to Fig. 3A hints that species-specific mRNA destabilization might also be frequent, presumably as both neutral and consequential species-specific targeting.

Quantifying selective depletion of sites among messages preferentially expressed in muscle indicated that ~420 of the 8511 genes of the expression atlas are under selective pressure to avoid miR-133 sites. These are "antitargets," an anticipated class of genes not observed previously (*14*). The estimated numbers of antitargets for miR-1, miR-122, miR-142, miR-9, and miR-124 were 300, 190, 170, 240, and 440, respectively—comparable to the numbers of their conserved targets. Extrapolating to include other miRNA families that are also highly expressed with specific spatial or temporal expression patterns, we estimated that selective avoidance of miRNA targeting extends to thousands of genes (SOM text). A signal for messages avoiding targeting in all tissue types would be harder to detect in our analysis. For some messages, acquiring miRNA pairing might be so detrimental that they are under selective pressure to have short UTRs, perhaps helping to explain why highly expressed "housekeeping" genes have substantially shorter UTRs than do other messages (*19*).

In addition to revealing target avoidance, these data extend results of our heterologous reporter system (Fig. 2) into the animal, showing that 7-nt sites are often sufficient to specify a biological effect. Messages expressed highly and specifically in muscle are ~59% less likely than controls to possess a 7-nt match to muscle-specific miR-133 (Fig. 3A). For the other five miRNAs, this depletion averaged 45% (range of 31 to 57%). This extent of depletion implies that as sites for highly expressed miRNAs emerge during sequence drift of UTRs, about half emerge in a context suitable for miRNA targeting—causing either mRNA destabilization or a selective disadvantage sufficient for preferential loss of the site from the gene pool.

Site depletion due to miRNA activity should occur specifically in tissue types expressing the miRNA. To explore the specificity of depletion, we used a modified Kolmogorov-Smirnov (KS) test to determine whether the set of genes with sites in either human or mouse orthologs were expressed at lower levels than cohorts of genes with the same estimated expectation for having sites, controlling for UTR length and nucleotide composition. In muscle, but not in T cells, the set of transcripts with a miR-133 site was depleted compared with those of control cohorts (Fig. 4A). Repeating the miR-133 analysis for all 61 tissues in the mouse atlas showed that this effect was most pronounced in skeletal muscle and heart, the two tissues in which miR-133 is preferentially expressed. Plotting color-coded P values for relative depletion of transcripts with miR-133 sites illustrated a signature reflecting the tissue-specific influence of miR-133 (Fig. 4B, top row).

Signatures for all 73 miRNA families (representing 169 human miRNA genes) conserved among the four sequenced mammals and zebrafish were derived (fig. S7). For many miRNA families that are prominently expressed in specific tissues (*7*–*10*), the signatures corresponded to tissues in which these miRNAs are expressed (Fig. 4B). These included the six families featured in Fig. 3, as well as let-7, miR-99, miR-29, and miR-153 (brain), miR-30 (kidney), miR-194 (liver, gut, and kidney), miR-141 and miR-200b (olfactory epithelium and gut), miR-96 (olfactory epithelium), and miR-375 (pituitary). Eight of these also gave accurate signatures when considering sites in the coding sequences rather than 3′UTRs (SOM text). miR-7 had the highest signal in the pituitary. This miRNA is known to be prefer-

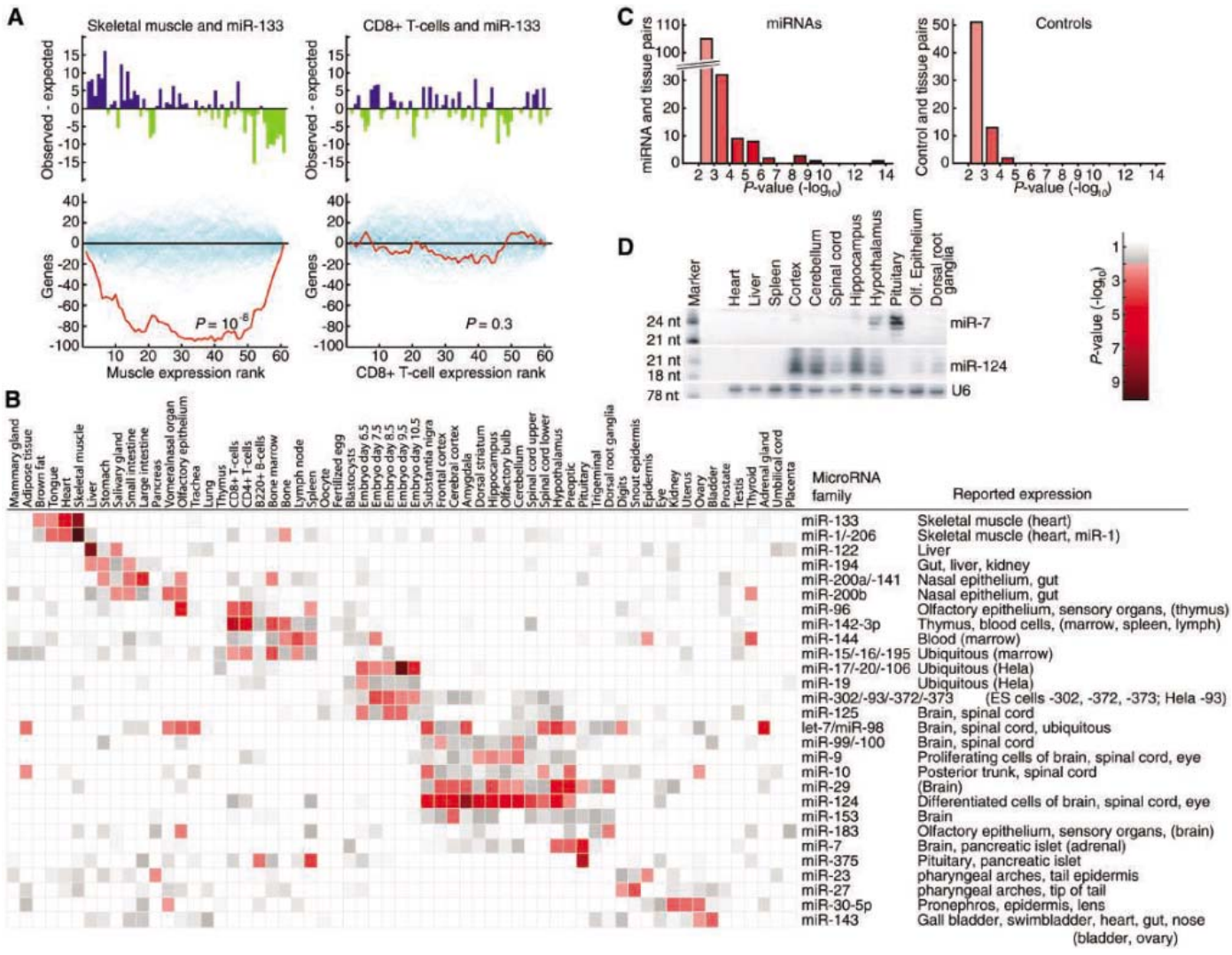

Fig. 4. Depletion of sites in genes preferentially coexpressed with the miRNA. (**A**) miR-133 sites in skeletal muscle and CD8+ T cells. For each panel, genes were binned based on their expression in the indicated tissue compared with expression in the 60 other tissues, with bin 1 lowest and bin 61 highest. (Top) Difference between observed and expected number of messages with miR-133 sites at each expression rank. (Bottom) Modified KS test and estimate of significance, showing the running sum of the difference between the observed and expected distributions across expression ranks for messages with sites (red) compared to control cohorts (blue). (**B**) Summary map of KS tests for each miRNA-tissue pair for 28 miRNAs; *P*-value key is shown above. Reported expression is from zebrafish in situ data (*10*), supplemented with notable mammalian data (*8*, *9*) (parentheses). ES cells, embryonic stem cells. (**C**) Tail of *P*-value distribution for all 73 miRNA families (left) (fig. S7) and for a mock analysis using control sequences (right). *P* values greater than 10^{-2}, which are gray in (B), were only marginally less frequent for controls. (**D**) RNA-blot analysis of miR-7 in rat tissues, reprobed for miR-124 and U6 small nuclear RNA.

entially expressed in the brain (*8*–*10*), but preferential expression in pituitary had not been noted. An RNA blot confirmed that miR-7 expression is highest in the pituitary (Fig. 4D).

Other miRNA families, including most described as having ubiquitous, complex, or undetectable expression patterns, were indistinguishable from controls (Fig. 4C and fig. S7). Nonetheless, some described as ubiquitous displayed stage-specific signatures. These included families in the miR-17~18~19a~20~19b~92 cluster, which had a strong embryo signature, consistent with their association with proliferation and cancer (*20*, *21*). The miR-302 family also had a strong early-embryo signature, consistent with its sequence similarity to the 17~92 proliferation cluster and its expression in embryonic stem cells (*22*, *23*). The conserved targets of these embryonic miRNAs were preferentially at high levels in the oocyte and zygote and then dropped to low levels in the blastocyst and embryo (Fig. 1C), as expected if these miRNAs help dampen expression of maternal transcripts.

A signal for motif conservation is a mainstay of bioinformatics and previously indicated the widespread scope of conserved miRNA targeting (*3*–*5*, *24*), but a signal for absence of a motif is unusual. The ability to observe such a signal revealed an additional dimension to the impact of miRNAs on UTR evolution—a widespread potential for nonconserved targeting leading to the selective loss of many 7-nt sites. When considering conserved targeting, nonconserved targeting, and targeting avoidance, it is hard to escape the conclusion that miRNAs are influencing the expression or evolution of most mammalian mRNAs.

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Ubiquitin-Binding Domains in Y-Family Polymerases Regulate Translesion Synthesis

Marzena Bienko,[1] Catherine M. Green,[2] Nicola Crosetto,[1]* Fabian Rudolf,[3]* Grzegorz Zapart,[1] Barry Coull,[2]† Patricia Kannouche,[2]‡ Gerhard Wider,[4] Matthias Peter,[3] Alan R. Lehmann,[2] Kay Hofmann,[5] Ivan Dikic[1]§

Translesion synthesis (TLS) is the major pathway by which mammalian cells replicate across DNA lesions. Upon DNA damage, ubiquitination of proliferating cell nuclear antigen (PCNA) induces bypass of the lesion by directing the replication machinery into the TLS pathway. Yet, how this modification is recognized and interpreted in the cell remains unclear. Here we describe the identification of two ubiquitin (Ub)–binding domains (UBM and UBZ), which are evolutionarily conserved in all Y-family TLS polymerases (pols). These domains are required for binding of polη and polι to ubiquitin, their accumulation in replication factories, and their interaction with monoubiquitinated PCNA. Moreover, the UBZ domain of polη is essential to efficiently restore a normal response to ultraviolet irradiation in xeroderma pigmentosum variant (XP-V) fibroblasts. Our results indicate that Ub-binding domains of Y-family polymerases play crucial regulatory roles in TLS.

Signaling through ubiquitin (Ub) is generally thought to occur by low-affinity noncovalent interactions between Ub and a variety of specialized Ub-binding domains (UBDs) (*1*, *2*). To analyze the Ub-interaction map, we performed yeast two-hybrid screens using wild-type Ub and Ub in which isoleucine 44 (I44) was mutated to alanine (Ub*). To date, all known characterized UBDs require the conserved I44 in the hydrophobic patch on Ub for their binding (*2*), and proteins interacting with Ub* might therefore contain previously unknown Ub-interacting modules. Among the clones that interacted with Ub* are two that encode the C terminus of TLS polymerase ι (polι) (fig. S1A). Moreover, full-length mouse polι expressed in human embryonic kidney (HEK) 293T cells bound to both glutathione *S*-transferase (GST)–Ub and GST-Ub*, but not to GST alone (fig. S1A). Thus, polι contains a Ub-binding module in the C terminus that does not require I44 for its binding to Ub. Bioinformatic analysis of the C-terminal part of polι identified two copies of a previously unknown sequence motif termed UBM (Ub-binding motif). These repeats span ~30 residues and consist of two predicted helical segments, separated by an invariant "Leu-Pro" motif, which is conserved in all polι versions examined, as well as in Rev1, another Y-polymerase (fig. S1B). Missense mutations of the conserved residues with a presumptive crucial role in Ub binding (L508A, P509A in UBM1*, L693A, P694A in UBM2*) in either polι UBM substantially impaired polι binding to GST-Ub, whereas the inactivation of both domains by point mutations completely blocked the interaction (Fig. 1A). Similar results were obtained using polι UBM deletion (polι-Δ496-524 and polι-Δ681-709) mutants (fig. S1C). We purified isolated GST-UBM1 and GST-UBM2 of polι and analyzed their binding to Ub and the Ub-I44A mutant by nuclear magnetic resonance (NMR) spectroscopy (fig. S1D). The estimated dissociation constant (K_d) values for binding of UBM1 and UBM2 to both Ub and Ub-I44A were in the range of 180 μM. Mapping of the

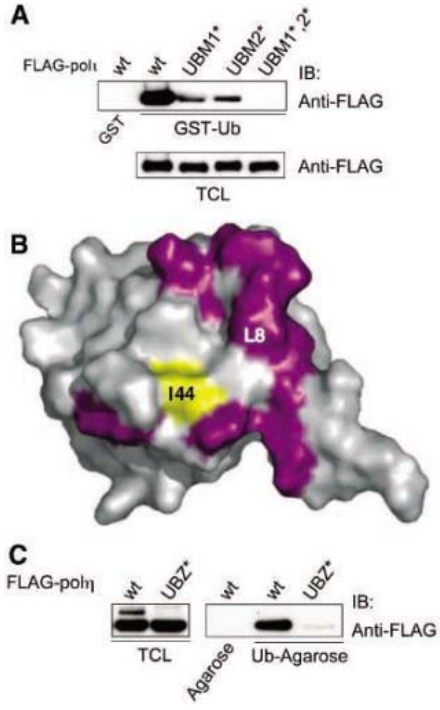

Fig. 1. (**A**) Identification of the UBDs in Y-polymerases. Point mutations of either UBM1 (L508A,P509A in UBM1*) or UBM2 (L693A,P694A in UBM2*) of mouse polι reduce its binding to Ub as compared with wild-type polι (wt). Mutating both UBMs (UBM1*,2*) abolishes binding of polι to Ub in GST pull-down assays. (**B**) Surface representation of Ub interaction with UBM determined by NMR spectroscopy. The binding interface of GST-UBM2 on Ub defined by residues K6, L8, T9, G10, I13, T14, R42, K48, G53, and R72 (see supporting online material) is indicated in purple. Residue I44 (yellow) is indicated for orientation. (**C**) Polη UBZ mediates binding to ubiquitin. HEK293T lysates (TCL) containing FLAG-polη wild type or its UBZ mutant (D652A) (UBZ*) were subjected to Ub-agarose pull-down assays. The shift in mobility of polη visible in lane 1 represents its monoubiquitinated form. IB, immunoblot.

[1]Institute for Biochemistry II, Goethe University Medical School, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany. [2]Genome Damage and Stability, University of Sussex, Falmer, Brighton BN1 9RQ, UK. [3]Institute of Biochemistry, [4]Institute of Molecular Biology and Biophysics, ETH Hönggerberg, 8093 Zürich, Switzerland. [5]Bioinformatics Group, Miltenyi Biotec GmbH, Stoeckheimer Weg 1, D-50829 Koeln, Germany.

*These authors contributed equally to this work.
†Present address: Life Sciences, Unilever R&D, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK.
‡Present address: Laboratory of Genetic Instability and Cancer, CNRS, Institut Gustave Roussy, 94805 Villejuif, France.
§To whom correspondence should be addressed. E-mail: ivan.dikic@biochem2.de

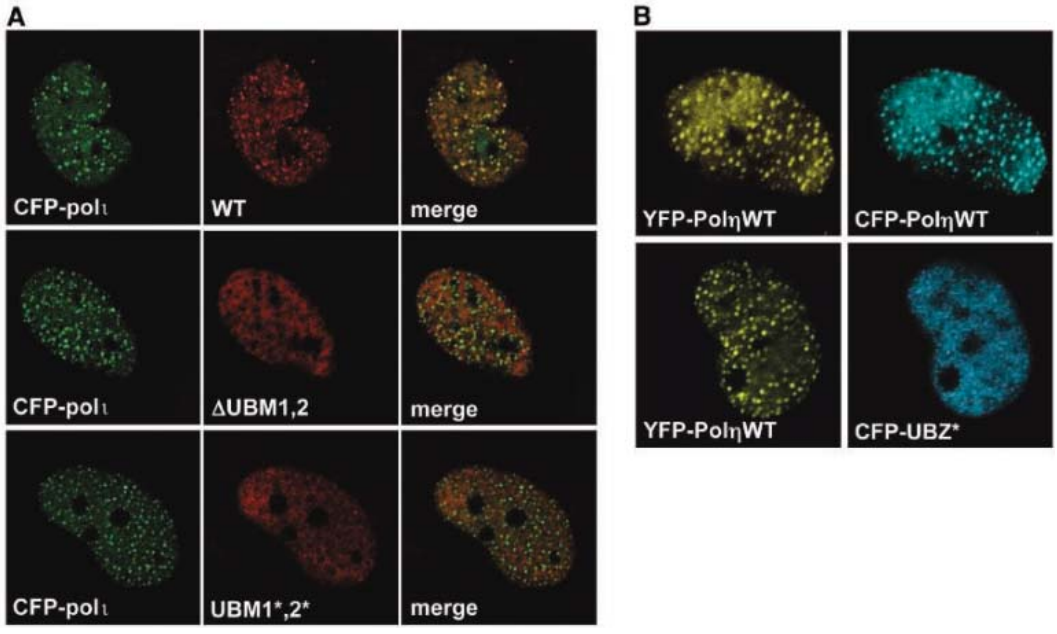

Fig. 2. UBMs and UBZ are essential for the accumulation of polι and polη in replication foci. (**A**) MRC5 fibroblasts were cotransfected with pECFP–polι wild type (left panels) and pCMV-FLAG polι, either wild type (WT) or mutants, as indicated (middle panels). The cells were UV irradiated with 15 J m^{-2} and fixed 16 hours later. (**B**) MRC5 fibroblasts were cotransfected with YFP-polη wild type and CFP-polη, either wt or D652A, and treated as in (A).

UBM2 binding surface on Ub revealed binding around the previously defined hydrophobic patch, but the binding surface is displaced toward L8 and away from I44 (Fig. 1B).

Apart from the UBMs, we also identified several yeast two-hybrid clones containing mononucleate Zn fingers, which were required for their binding to Ub in yeast and mammalian cells. Using profile-based sequence comparisons (*3*, *4*), we grouped these Ub-binding Zn fingers into a separate family, which we named UBZ (Ub-binding Zn finger). These sequence profile searches showed that the UBZ-family Zn fingers can be clearly separated from the presumed DNA-binding variety and are completely unrelated to PAZ (polyUb-associated Zn finger) (*5*) and NZF (Npl4 Zn finger) (*6*). UBZ-type fingers were also found in the remaining two Y-family polymerases polη and polκ (fig. S1E). Indeed, human polη is a Ub-binding protein because it interacted specifically with monoUb coupled to agarose (Fig. 1C), and point mutation of the conserved aspartate 652 to alanine in the UBZ (UBZ*) prevented the interaction of polη with monoUb. Thus, all members of the Y-family polymerases contain UBDs in their C termini (fig. S1F).

Polι and polη colocalize with each other and with proliferating cell nuclear antigen (PCNA) in replication factories. These appear as bright foci in S-phase cells, which accumulate upon ultraviolet (UV) irradiation (*7*, *8*). To examine whether this localization requires their UBDs, we cotransfected MRC5 fibroblasts with wild-type cyan fluorescent protein (CFP)–polι and wild-type or mutated FLAG-polι. The fraction of cells with foci increased in UV-irradiated cells to ~60%, and as expected, both wild-type constructs colocalized in foci. However, neither ΔUBM1,2 nor UBM1*,2* mutants of polι localized in replication foci (Fig. 2A). Furthermore, the *Xenopus tropicalis* polι, which contains two UBMs but few other conserved amino acids in the C-terminal part (fig. S2A), bound to Ub (fig. S2B) and localized in replication factories in human cells (fig. S2C).

In similar cotransfection experiments, yellow fluorescent protein (YFP)- or CFP-tagged wild-type polη formed bright foci in S-phase cells accumulated upon UV irradiation, whereas CFP-polη−D652A mutant (UBZ*) formed very faint or no foci (Fig. 2B). We conclude that foci localization of both polη and polι depends on their ability to interact with Ub, indicating a common mechanism for the accumulation of Y-polymerases in replication foci. Notably, the polymerase activity was identical in wild-type and mutant proteins used (fig. S3A).

Ubiquitination of the polymerase processivity factor PCNA controls switching of polymerases and replication of damaged DNA (*9*–*13*). TLS polymerases polη and polι bind directly to PCNA via their PCNA-interacting peptide (PIP box) (*11*, *14*–*16*). In addition, DNA damage–induced ubiquitination of PCNA on K164 increases its interaction with polη in vivo (*11*, *13*). We therefore investigated whether the UBM domains of polι directly bind to monoubiquitinated PCNA upon DNA damage. Isolated UBMs of polι readily precipitated monoubiquitinated PCNA generated in HEK293T cells by hydroxyurea treatment (Fig. 3A). UBMs also interacted directly with monoubiquitinated His-PCNA and with a permanently ubiquitinated PCNA, engineered by fusing the cDNA for Ub in frame with that for His-PCNA-K164R mutant (PCNA*-Ub chimera) (fig. S3B). We next analyzed whether simultaneous binding of PIP and UBM domains of polι to ubiquitinated PCNA enhanced this interaction in vivo. Coprecipitation of polι and unmodified PCNA from cells was hardly detectable, whereas the PCNA*-Ub chimera strongly bound to polι in transiently transfected HEK293T cells (Fig. 3B). Coprecipitation was substantially reduced when the PIP box was mutated in polι and completely abolished if UBM domains or both PIP box and UBM domains of polι were mutated.

We next examined the importance of the UBDs in polη for its functions in vivo. Xeroderma pigmentosum variant (XP-V) patients are defective in polη, resulting in elevated levels of UV mutagenesis and skin cancers (*17*). XP30RO is an XP-V fibroblast line that is sensitive to UV irradiation in the presence of caffeine (*17*). Transfection of green fluorescent protein (GFP)–polη into XP30RO cells restores the UV plus caffeine sensitivity to that of normal human fibroblasts (Fig. 3C). However, when we transfected a UBZ mutant of polη (C638A) into XP30RO cells, the ability to confer resistance to UV plus caffeine treatment was substantially impaired. A reduction in this ability, though less marked, was also obtained with the PIP box mutation. Thus, both Ub- and PCNA-binding abilities of polη are required for efficient TLS. In previous work, we identified a motif in the little finger domain of polη that was required for its interaction with ubiquitinated PCNA; we thought this motif was similar to a CUE domain (*11*). Current bioinformatic and biochemical studies indicate that this motif is not a bona fide UBD. It is likely therefore that it is involved in the polη-PCNA interaction, irrespective of ubiquitination status.

UBDs can mediate monoubiquitination of the proteins containing them (*1*, *2*). When FLAG-polι was transfected into HEK293T

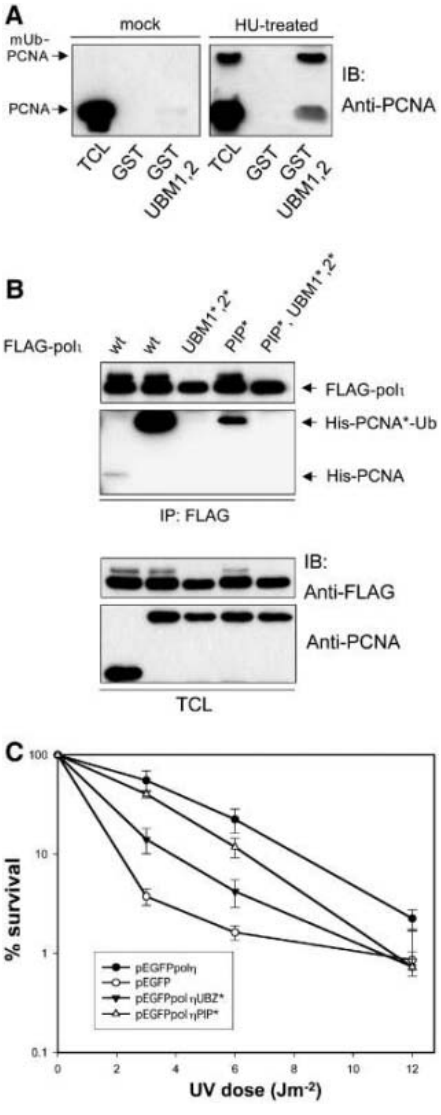

Fig. 3. Dual mode of polι-monoUb-PCNA interaction. (**A**) The UBM domains of polι mediate interaction with monoubiquitinated PCNA (mUb PCNA) generated in HEK293T cells by hydroxyurea (HU) treatment. Total cell lysates or proteins bound to GST or GST-UBMs were analyzed by immunoblotting (IB) with antibodies to PCNA (anti-PCNA). A small amount of nonubiquitinated PCNA precipitated with GST-UBM domains, presumably owing to heterotrimerization of ubiquitinated monomers with nonubiquitinated ones. (**B**) Both the UBM and PIP box motifs of polι mediate its binding to PCNA*-Ub chimera. HEK293T cells were transfected with His-PCNA*-Ub and FLAG-polι (wt) or its PIP* mutant (FLAG-polι-D424A/C425A/Y426A), UBM1*,2* mutant, or PIP*,UBM1*,2* mutant. The TCL shows the expression level of the corresponding proteins that were subsequently subjected to anti-FLAG immunoprecipitation (IP). Immunoprecipitated polι and PCNA*-Ub were detected with anti-FLAG and anti-PCNA, respectively. (**C**) XP30RO XP-V cells were transfected with pEGFP-polη constructs, and stable clones were isolated. The survival of these clones was measured after exposure to different doses of UV irradiation and plating in the presence of caffeine (75 μg/ml). UBZ* represents the C638A mutation.

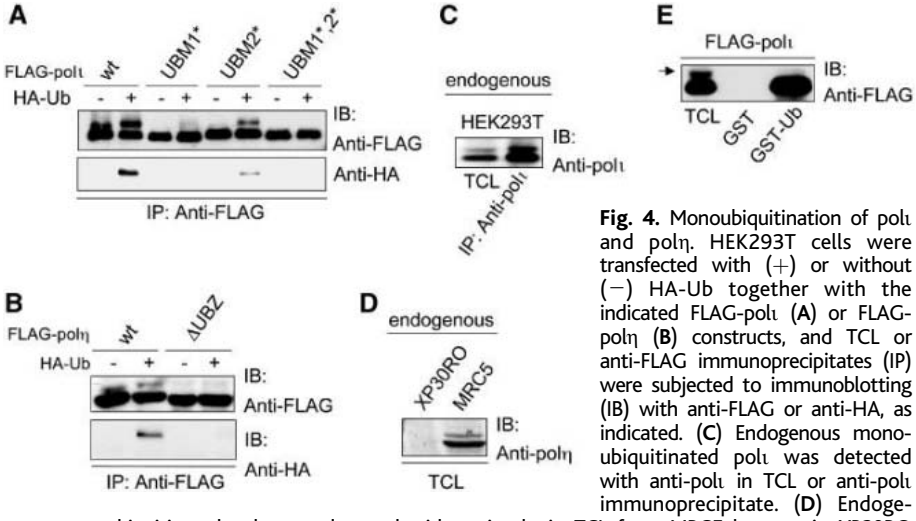

Fig. 4. Monoubiquitination of polι and polη. HEK293T cells were transfected with (+) or without (−) HA-Ub together with the indicated FLAG-polι (**A**) or FLAG-polη (**B**) constructs, and TCL or anti-FLAG immunoprecipitates (IP) were subjected to immunoblotting (IB) with anti-FLAG or anti-HA, as indicated. (**C**) Endogenous monoubiquitinated polι was detected with anti-polι in TCL or anti-polι immunoprecipitate. (**D**) Endogenous monoubiquitinated polη was detected with anti-polη in TCL from MRC5 but not in XP30RO cells (**E**) HEK293T lysates containing FLAG-polι were subjected to pull-down assays with GST-Ub, and bound proteins were detected by immunoblotting with anti-FLAG. The arrow indicates the monoubiquitinated form of polι.

cells, a band of polι of reduced mobility was detected by immunobloting with antibodies to FLAG (Fig. 4A). Analysis of cells additionally transfected with hemagglutin (HA)–Ub showed that the protein of reduced mobility was monoubiquitinated polι (Fig. 4A). Under similar conditions, we found a species of polη with reduced mobility (Fig. 4B; see also Fig. 1C), which we showed was monoubiquitinated polη (Fig. 4B). Mutational inactivation of UBMs in polι or UBZ in polη abolished their monoubiquitination (Fig. 4, A and B). We also detected endogenous levels of monoubiquitinated forms of both polymerases (Fig. 4, C and D). The monoubiquitinated species of polι appeared to be no longer capable of binding to Ub. In GST-Ub pull-down assays, only the unmodified form of polι was detected (Fig. 4E; compare lane 3 with lane 1). Similarly, only the unmodified form of polη bound to Ub-agarose beads (Fig. 1C). In accordance with these observations, creating a permanently ubiquitinated form of polη by fusing Ub to the C terminus of polη (polη-Ub chimera) strongly reduced its ability to bind to Ub-agarose beads (fig. S3C). These results indicate that monoubiquitinated polymerases might be blocked in binding to Ub because of autoinhibitory interactions with their own UBDs (*18*).

In conclusion, we have identified two previously unknown UBDs in the Y-family TLS polymerases that enable them to interact with monoubiquitinated targets and undergo monoubiquitination in vivo. UBDs are critical for accumulation of polι and polη in replication foci in human cells and are required for efficient restoration of normal TLS in XP-V cells. Both polι (Fig. 3, A and B) and polη (*11*, *13*) preferentially interact with monoubiquitinated PCNA, which is generated at stalled replication forks (*11*, *13*). The PIP box provides the specificity for the interaction, and the DNA damage–induced conjugation of a Ub moiety to PCNA increases the avidity of this binding by providing an interaction surface for the UBDs. We have also shown that polι and polη are themselves monoubiquitinated in vivo. Although the precise role of monoubiquitination of the polymerases remains to be established, it is tempting to speculate that a cycling between their nonubiquitinated and monoubiquitinated forms may contribute to regulation of their compartmentalization in or out of replication factories. Taken together, our data show that Ub binding of the Y-family polymerases plays an important role in translesion DNA synthesis and provide a long-sought clue to how these polymerases can gain preferential access to the stalled replication machinery at the sites of DNA damage.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5755/1821/DC1

Materials and Methods
Figs. S1 to S3
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Chitin Induces Natural Competence in *Vibrio cholerae*

Karin L. Meibom,*† Melanie Blokesch,* Nadia A. Dolganov, Cheng-Yen Wu, Gary K. Schoolnik†

The mosaic-structured *Vibrio cholerae* genome points to the importance of horizontal gene transfer (HGT) in the evolution of this human pathogen. We showed that *V. cholerae* can acquire new genetic material by natural transformation during growth on chitin, a biopolymer that is abundant in aquatic habitats (e.g., from crustacean exoskeletons), where it lives as an autochthonous microbe. Transformation competence was found to require a type IV pilus assembly complex, a putative DNA binding protein, and three convergent regulatory cascades, which are activated by chitin, increasing cell density, and nutrient limitation, a decline in growth rate, or stress.

Rivers, estuaries, and coastal waters are the principal reservoir for *Vibrio cholerae* in nature. In habitats of this kind, *V. cholerae* is found as a planktonic organism in the water column, in the mucilaginous sheaths of blue-green algae, and on the chitinous exoskeletons and molts of copepods (*1*). Population structure studies of aquatic habitats typically disclose ecosystems containing multiple microbial strains and species and high concentrations of phage and free DNA (*2*, *3*). These features, when combined with mechanisms for HGT, likely explain why the *Vibrionaceae* have developed high levels of genomic diversity (*4*, *5*).

One microenvironment where HGT could occur between *V. cholerae* and other strains and species is within microbial assemblages on natural chitin surfaces. *V. cholerae* readily attaches to and degrades chitin, a polymer of β-1,4–linked *N*-acetylglucosamine (GlcNAc). Chitin induces the expression of a 41-gene regulon involved in chitin colonization, digestion, transport, and assimilation, including genes predicted to encode a type IV pilus assembly complex (*6*).

V. cholerae has never been shown to be competent for natural transformation, and thus, with respect to HGT events, its genome is presumed to have evolved by transduction (responsible for the acquisition of the *ctx* genes encoding cholera toxin) and conjugation (*7*, *8*). However, the induction of type IV pilin by chitin and the association of type IV pili and competence in several other species led us to test if chitin might induce natural competence in *V. cholerae* (*9*). We grew *V. cholerae* O1 El Tor, strain A1552, in a liquid minimal medium containing 2.5 mM $(GlcNAc)_6$, a soluble chitin oligosaccharide that induces the chitin regulon (*6*). Then, genomic DNA from the *V. cholerae* O1 El Tor strain VCXB21, which harbors a gene for kanamycin resistance on the chromosome, was added to the culture. After 18 hours of growth, the culture was plated onto antibiotic-free and kanamycin-containing LB agar; this yielded a transformation frequency [kanamycin-resistant (Kn^r) colony-forming units (CFU)/

Division of Infectious Diseases and Geographic Medicine, Department of Microbiology and Immunology, and Stanford Institute for the Environment, Stanford University, Stanford, CA 94305, USA.

*These authors contributed equally to the work.
†To whom correspondence should be addressed. E-mail: kmeibom@necker.fr (K.L.M.); schoolni@cmgm.stanford.edu (G.K.S.)

Table 1. Transformation of *V. cholerae*; data are the average of at least three experiments. Transformation frequency is Kn^r or Str^r CFU/total CFU; <DL, below detection limit (for values in (A), $\sim 4.0 \times 10^{-8}$; for (B), $\sim 3.0 \times 10^{-9}$; for (C), $\sim 1.0 \times 10^{-7}$; for (D), $\sim 4.0 \times 10^{-8}$).

Donor DNA	Recipient strain	Medium	Transformation frequency	Range
A. Transformation in liquid medium				
VCXB21	A1552	+ $(GlcNAc)_6$	2.7×10^{-5}	1.4 to 6.8 $\times 10^{-5}$
VCXB21	A1552	+ $(GlcNAc)_6$ + DNase	<DL	
None	A1552	+ $(GlcNAc)_6$	<DL	
VCXB21	A1552	+ $(GlcNAc)_6$ + Glucose	<DL	
VCXB21	A1552	+ Glucose	<DL	
VCXB21	A1552	+ GlcNAc	<DL	
B. Transformation in liquid medium of His and Pro auxotrophs				
VCXB21	A1552*proC*	+ $(GlcNAc)_6$	2.7×10^{-6}	4.0×10^{-7} to 1.1×10^{-5}
None	A1552*proC*	+ $(GlcNAc)_6$	<DL	
VCXB21	A1552*hisD*	+ $(GlcNAc)_6$	6.8×10^{-6}	4.0×10^{-8} to 1.7×10^{-5}
None	A1552*hisD*	+ $(GlcNAc)_6$	<DL	
C. Transformation of chitin surface–attached bacteria				
VCXB21	A1552	Crab shell	3.5×10^{-5}	5×10^{-6} to 6.9×10^{-5}
None	A1552	Crab shell	<DL	
N16961	A1552	Crab shell	1.8×10^{-5}	5×10^{-6} to 4.4×10^{-5}
VCXB21	A1552	Crab shell + DNase	3.7×10^{-7}	<DL to 8.0×10^{-7}
VCXB21	N16961	Crab shell	<DL	
VCXB21	C6706	Crab shell	2.8×10^{-6}	8.0×10^{-7} to 5×10^{-6}
VCXB21	0395 (classical)	Crab shell	<DL	
D. Transformation in biofilm communities without exogenous DNA				
None	A1552 -Kn/-Str	Crab shell	4.4×10^{-5}	1.4 to 8 $\times 10^{-5}$
None	A1552 -Kn/-Str	Crab shell + DNase	$<1.2 \times 10^{-7}$	<DL to 1.2×10^{-7}

total CFU] of 2.7×10^{-5} (Table 1). In the absence of donor DNA or when deoxyribonuclease (DNase) and donor DNA were added simultaneously, no Kn^r colonies were detected. Of other carbohydrates tested, including the chitin monomer GlcNAc, which does not upregulate the chitin regulon, only chitin induced the competence phenotype. When glucose was combined with $(GlcNAc)_6$, competence was inhibited, which suggests that the competence phenotype is subject to catabolite repression. Chitin-induced natural transformation with genomic DNA from the prototroph strain VCXB21 also restored prototrophy to two amino acid auxotrophic mutants that had deletions in either the *proC* or *hisD* gene and, thus, were unable to synthesize proline or histidine (Table 1). That the deleted version of the *hisD* gene was replaced by the wild-type copy from the donor DNA was shown by polymerase chain reaction (PCR) (fig. S1) and is indicative of homologous recombination. Together, these experiments showed that the growth of *V. cholerae* O1 with a soluble chitin oligosaccharide induced transformation competence and the capacity to acquire different genetic markers.

In nature, *V. cholerae* experiences chitin as an insoluble polymer provided as a structural component of copepod exoskeletons and molts on which it grows as surface-attached colonies or as a biofilm (*1*). To determine whether growth on a natural chitin surface induced the competence phenotype, strain A1552 was allowed to establish a surface-attached population on a sterile crab shell fragment in defined (artificial) seawater medium (DASW) for 24 hours. Then, the crab shell was immersed in fresh DASW containing antibiotic-marked genomic DNA from *V. cholerae* O1 strain VCXB21 (Kn^r) or strain N16961, which is streptomycin-resistant (Str^r). These experiments yielded transformation frequencies of 3.5×10^{-5} and 1.8×10^{-5}, respectively; in the absence of added DNA, no transformants were obtained (Table 1). The addition of DNase reduced transformation frequency ≥100-fold, but did not abolish it entirely, possibly because of the resistance of surface-adsorbed DNA to DNase (*10*) or to the use of conditions in the transformation assay that are not optimal for the activity of this enzyme. Thus, during growth on a chitin surface, *V. cholerae* becomes competent for transformation by genomic DNA from other *V. cholerae* strains.

Some naturally competent species release DNA to an extent that can lead to localized concentrations in a biofilm that exceed 100 μg/ml (*11*). Simultaneously, transformation competence develops among other members of the population (*12*, *13*). To determine whether *V. cholerae* can be transformed when DNA is provided by other members of a surface-attached consortium, two variants of the same competent *V. cholerae* strain (A1552), with different antibiotic resistance markers on the chromosome (-Kn^r or -Str^r), were propagated together on a crab shell fragment (*9*). Twenty-four hours later, planktonic bacteria were discarded and fresh DASW added; 24 hours thereafter, the bacteria were detached from the crab shell surface and plated onto LB agar and LB agar containing both Kn and Str, then the colonies were counted. Experimental replicates yielded transformants resistant to both antibiotics with an average frequency of 4.4×10^{-5} (Table 1). The addition of DNase to the crab shell culture reduced transformation efficiency by ≥100-fold (Table 1). Thus, naked DNA is apparently provided in situ by *V. cholerae* growing on a chitin surface and can be acquired by competent members of the consortium.

Twelve chitin-induced genes are predicted to encode components of a type IV pilus assembly complex (*6*). To determine whether a type IV pilus is required for competence, the following genes were disrupted in *V. cholerae* strain A1552: *pilA* (VC2423), predicted to encode a type IV pilin; *pilQ* (VC2630), encoding a homolog of the secretin protein family; and *pilB* (VC2424), which specifies a traffic nucleotide triphosphatase(NTPase) believed to energize assembly of the pilus filament. The wild-type parent and each of the mutants were tested for competence with the crab shell transformation assay and DNA from the Kn^r strain VCXB21. The wild-type parent exhibited a transformation frequency of 3.5×10^{-5}; no Kn^r transformants were detected for any of the mutants (Table 2). Thus, at least three components of a putative type IV pilus assembly complex are required for competence. This finding prompted us to use transmission and scanning electron microscopy (TEM and SEM, respectively) to search for new or additional pilus filaments emanating from the surface of chitin-induced bacteria. None was identified, which indicates that this assembly complex likely directs the synthesis of a competence pseudopilus (*14*).

We used BLAST to search among the 41 chitin-induced genes for homologs of competence genes in other species. One of these, VC1153 (further named *tfoX^{VC}*), was found to contain two TfoX domains, which are involved with competence regulation in *Haemophilus influenzae* (*15*, *16*). To determine whether *tfoX^{Vc}* is required for competence, it was disrupted in *V. cholerae* strain A1552, and the mutant was tested for competence by using the crab shell transformation assay. No transformants were detected (Table 2). To determine whether *tfoX^{Vc}* expression would promote transformation competence in the absence of chitin, *tfoX^{Vc}* was placed under the control of an arabinose-inducible promoter, and then either the vector alone (pBAD) or the recombinant plasmid (pBAD-*tfoX*) was introduced into the A1552*tfoX* mutant. Growth of the complemented mutant in LB liquid medium containing arabinose (but no chitin) led to chitin-independent overexpression of *tfoX^{Vc}*. The addition of genomic DNA from *V. cholerae* strain VCXB21 to this culture yielded Kn^r transformants at a frequency of 2.9×10^{-6}. By contrast, under the same growth conditions, neither the wild-type parent nor the A1552*tfoX* mutant was competent (Table 3).

The demonstration that TfoX^{Vc} is required for competence and the role of its ortholog in *H. influenzae* as a regulator of competence prompted us to use microarray expression profiling to identify genes it might regulate in *V. cholerae*. Transcriptional profiles were obtained by using RNAs isolated from the

Table 2. Transformation of *V. cholerae* O1 El Tor strains and mutants in a crab shell–associated community. Transformation with DNA from *V. cholerae* strain VCXB21; data are the average of at least three experiments. Transformation frequency is Kn^R CFU/total CFU; <DL: below detection limit (1.0×10^{-7}). Similar results were obtained using liquid medium with $(GlcNAc)_6$ as carbon source.

Strain	Genotype	Transformation frequency
A1552	O1 El Tor, wt	3.5×10^{-5}
A1552*pilA*	A1552ΔVC2423	<DL
A1552*pilB*	A1552ΔVC2424	<DL
A1552*pilQ*	A1552ΔVC2630	<DL
A1552*tfoX*	A1552ΔVC1153	<DL
A1552*VC1917*	A1552ΔVC1917	<DL
FY1	A1552Δ*rpoS*	<DL
ATN140	A1552Δ*hapR*	<DL
ATN194	A1552Δ*hapR*::mTn*7hapR*	1.3×10^{-5}
N16961	O1 El Tor	<DL
N16961*ChapR*	N16961::mTn*7hapR*	7.5×10^{-6}

Table 3. Expression of *tfoX^{VC}* allows transformation in the absence of chitin. Transformation with DNA from strain VCXB21 in LB media supplemented with ampicillin and 0.2% L-arabinose; data are the average of two independent experiments. Transformation frequency, Kn^r CFU/total CFU; <DL: below detection limit (2.0×10^{-8}).

Strain	Plasmid	Transformation frequency
A1552	pBAD	<DL
A1552*tfoX*	pBAD	<DL
A1152*tfoX*	pBAD-*tfoX*	2.9×10^{-6}

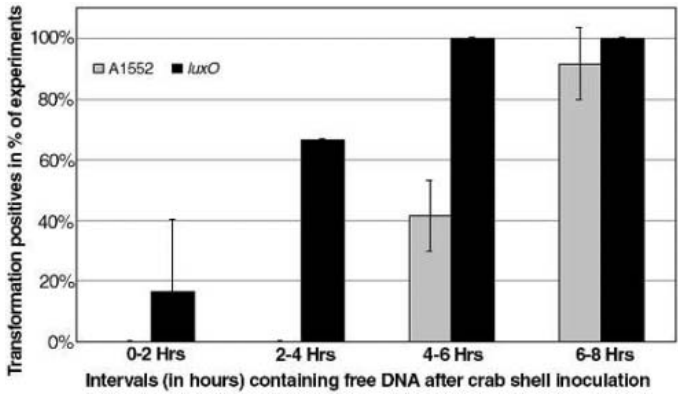

Fig. 1. Transformation frequency as a function of cell density. Transformation was scored as positive or negative for the crab shell–associated wild type (gray bars) or *luxO* mutant (black bars) of strain A1552 (9). Donor DNA from strain VCXB21 was present during the postinoculation time intervals indicated on the *x* axis. Transformation (*y* axis) is calculated as the percentage of experiments that were scored as transformation-positive, based on results from two replicates of six independent experiments.

A1552*tfoX* mutant described above, which harbors either pBAD (does not express $tfoX^{Vc}$) or pBAD-*tfoX* (arabinose-induced expression of $tfoX^{Vc}$) during mid log phase growth in LB liquid medium containing arabinose, but no chitin. Under these conditions, 99 genes were significantly and ≥2.5-fold induced in the culture expressing $tfoX^{Vc}$ compared with the control culture (table S1). Among the $TfoX^{Vc}$-induced genes were 28 that were previously reported to be induced by chitin, including the three pilus assembly genes, *pilA*, *pilB*, and *pilQ*; genes encoding four chitinases (including ChiA-1 and ChiA-2); and a chitoporin gene. Thus, $TfoX^{Vc}$ is induced by chitin and controls the expression of genes encoding proteins with two quite different functions: chitin degradation and chitin-induced competence.

The foregoing result encouraged us to search for other $TfoX^{Vc}$-regulated genes that might be required for competence. This led to the identification of VC1917, predicted to encode a protein with a signal peptide and a motif homologous to the DNA-binding helix-hairpin-helix domain found in the *Bacillus subtilis* ComEA protein (*17*). When VC1917 was disrupted and the mutant tested for competence, no transformants were obtained (Table 2). This shows that VC1917 is required for competence in *V. cholerae*.

During the course of this study, we tested a total of eight *V. cholerae* strains for transformation competence: four *V. cholerae* O1 strains and four recent *V. cholerae* non-O1 environmental isolates. Strain C6706, an El Tor biotype, and each of the environmental isolates exhibited chitin-dependent competence. By contrast, neither strain N16961, an O1 El Tor biotype for which a genome sequence is available, nor strain O395, a *V. cholerae* O1 classical biotype, was found to be competent under the same experimental condition (Table 1). Both of these nontransformable strains are reported to have a frameshift mutation in *hapR* (*18*), whose protein product coordinately down-regulates the expression of virulence determinants and biofilm formation and up-regulates hemagglutinin/protease (HA/protease) production in response to increasing cell density. To determine whether the reported frameshift mutation in *hapR* accounts for the competence-negative phenotype of strain N16961, it was complemented with a wild-type copy of *hapR* from the transformable strain A1552. When tested in the crab shell transformation assay, the complemented strain, N16961C*hapR*, showed a transformation frequency of 7.5×10^{-6} (Table 2). To further examine the requirement of *hapR* for the competence phenotype, it was disrupted in A1552. The resulting mutant was transformation-negative (Table 2). Together, these data show that HapR is required for transformation competence.

The expression of *hapR* is positively controlled by the alternative sigma factor RpoS (*19*, *20*). To find out if it is also required for transformation competence, an *rpoS* mutant was tested using the crab shell transformation assay and found to have a transformation-negative phenotype (Table 2). Thus, RpoS likely modulates transformation efficiency through its effect on *hapR* expression. The natural effectors of increased RpoS abundance in a chitin-associated biofilm were not identified. However, plausible candidates include nutrient limitation as population density increases, growth deceleration, or stress (*20*).

At low cell densities, *hapR* expression is repressed by the quorum-sensing regulator phospho-LuxO; at high cell densities, LuxO is dephosphorylated, the repression is relieved, and HapR is synthesized (*18*, *21*). Because HapR is required for competence, we tested the effect of cell density on the competence phenotype. *luxO* was disrupted in A1552 and the mutant compared with the wild-type strain using the crab shell transformation assay. The average transformation frequency of the *luxO* mutant was about five times that of the wild-type parent. We reasoned that the effect of a *luxO* mutation on transformation efficiency would be most evident at low cell densities. To correlate bacterial cell density with transformation frequency, genomic DNA from Kn^R strain VCXB21 was added at 0, 2, 4, or 6 hours after inoculation of the crab shell, time intervals that correspond to increasing cell densities on the crab shell surface. Two hours after the addition of genomic DNA, the assay was treated with DNase to degrade residual donor DNA. Then the culture was allowed to grow for a total of 24 hours, and the experiment was scored as transformation positive or negative (*9*). Kn^R transformants were first evident more than 4 hours after we inoculated the crab shell assay with the wild-type strain. By contrast, the *luxO* mutant was transformable at lower cell densities, with some transformants noted between 0 and 2 hours after inoculation; many more transformants were evident after 2 to 4 hours (Fig. 1). These results show that high cell density positively controls transformation competence by relieving LuxO-dependent repression of HapR synthesis.

The data presented in this report have led us to propose a model of the *V. cholerae* competence regulatory network that posits three controlling environmental determinants of this phenotype. These are the presence of chitin; increasing cell density; and, nutrient limitation, growth deceleration, or stress (fig. S2). Chitin, acting through $TfoX^{Vc}$, induces the expression of a competence pseudopilus, as well as genes required for the extracellular degradation and uptake of chitin. Increasing cell density, in combination with effectors of heightened RpoS abundance, strengthen the expression of *hapR*, shown here to be required for the positive regulation of competence. Remarkably, both HapR and $TfoX^{Vc}$ are required for the expression of the VC1917 gene (tables S1 and S2) and thus the three environmental determinants of competence converge in the regulation of this and perhaps other genes.

This model highlights the importance of natural competence, occurring in chitin-attached bacterial communities in the aqua sphere, as a powerful driver of the evolution of *V. cholerae*. It further suggests that environmental events giving rise to copepod blooms likely foster the rapid evolution of this pathogen.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5755/1824/DC1
Material and Methods
Figs. S1 and S2
Tables S1 to S3
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DIRECTOR, DIVISION OF CELL BIOLOGY AND BIOPHYSICS

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Department of Health and Human Services (DHHS)

The Challenge: The NIGMS Division of Cell Biology and Biophysics supports a significant research grant program seeking greater understanding of the structure and function of cells, cellular components, and the biological macromolecules that make up these components. The research ranges from traditional cell biology and biophysics to studies of single molecules and work in structural genomics and proteomics. The long-term goal of the Division is to find ways to prevent, treat, and cure diseases that result from disturbed or abnormal cellular activity. The division has three components: the Biophysics Branch, the Cell Biology Branch, and the Structural Genomics and Proteomics Technology Branch. The Institute is seeking a leader in this field to direct the Division of Cell Biology and Biophysics, to coordinate the division's efforts with other federal agencies and the broader scientific community, and to supervise a staff of 11. Information about the division is available at: http://www.nigms.nih.gov/About/Overview/CBB.htm

Position Requirements: Candidates must have an M.D., Ph.D., or equivalent degree and post-doctoral training in the fields relevant to the position. The ideal candidate will have:

- Broad knowledge of both cell biology and biophysics;
- A demonstrated record of leadership and accomplishment in activities beyond the candidate's own research program; and
- Experience in the management of programs and people.

The position will be filled under Title 42, offering a competitive salary commensurate with qualifications and experience, within the range of $125,304 to $175,700. A recruitment or relocation bonus may be available. Relocation expenses will be paid.

How to Apply: The official vacancy announcement is available at: http://www.nigms.nih.gov/about/job_vacancies.html. To be considered for this position, send to the address below a CV, bibliography, the names and contact information of four references, and a "vision statement," not to exceed three pages, that describes your vision for the fields of cell biology and biophysics, where you see the fields going over the next ten years, and the role that NIGMS should play in enabling necessary developments.

nigmsjobs@mail.nih.gov or FAX to 301-451-5686
Applications must be received by midnight on the closing date: February 15, 2006

You may contact Erin Bandak, Human Resources Specialist, with questions about this vacancy on 301-594-2324.

FLORIDA STATE UNIVERSITY

Florida State University College of Medicine
Department of Biomedical Sciences
The Jim and Betty Ann Rodgers Eminent Scholar Chair

The Florida State University College of Medicine invites applications and nominations for the Jim and Betty Ann Rodgers Eminent Scholar Chair, which carries the rank of Professor in the Department of Biomedical Sciences. The tenure-track position has been established to enhance the research programs of the department and the College of Medicine. The Florida State University College of Medicine is the first allopathic medical school to be established in the United States in 20 years (**http://med.fsu.edu**) and was created by the Florida Legislature for the purpose of training physicians devoted to the needs of rural, underserved, minority, and elderly populations.

The successful candidate for the Jim and Betty Ann Rodgers Chair in Biomedical Sciences must be:

- An internationally recognized distinguished leader in his/her field,
- Dedicated to a world-class research program with a continuously distinguished record of scholarship and research, including publications and funding,
- Able to develop a research group or institute in his/her field,
- Able to lead university and/or state-wide development of interdisciplinary programs,
- Able to foster national eminence of academic programs, so as to address critical state needs and meet the mission of the College of Medicine,
- Able to engage in the recruitment, education, training, and mentoring of other scholars, and
- Experienced with graduate programs and the administration of these programs.

The Department of Biomedical Sciences is the basic science research and teaching arm of the College of Medicine. The department has grown to more than 20 faculty in just four years and will add several more faculty by 2008. The second phase of our new research building, the first phase of which was occupied in November of 2004, will be completed by March 2006 giving the College of Medicine complex more than 300,000 sq. ft. including more than 150,000 sq. ft. of laboratory space. The Department of Biomedical Sciences has an extensive inventory of common use equipment and state-of-the art core labs in proteomics, genomics, confocal microscopy, flow cytometry, and cell culture. See **http://med.fsu.edu/biomed** for more information.

The Florida State University is recognized for outstanding research and graduate programs in many areas (e.g., National High Magnetic Field Laboratory, Neuroscience, Structural Biology) and has begun a major initiative to strengthen interdisciplinary research teams and recruit science faculty campus-wide. The environment for collaboration is excellent. Located in northern Florida, Tallahassee is a beautiful, mid-sized capital city with a mild climate and excellent cultural and recreational opportunities.

Applications and nominations for the Jim and Betty Ann Rodgers Eminent Scholar Chair should be mailed to: **Alma Littles, M.D., Chair, Jim and Betty Ann Rodgers Eminent Scholar Chair Selection Committee, The Florida State University College of Medicine, 1115 West Call Street, Tallahassee, FL 32306-4300**. Letters of nomination should include potential candidate's name, contact information and an explanation of qualifications for the position. Direct applications should include a complete CV and request for three (3) letters of reference. Consideration of nominees and applicants will begin on **February 1, 2006**.

The Florida State University is an Equal Opportunity/Affirmative Action Employer.

Director

Royal Botanic Gardens, Kew

Kew

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RBG Kew is the world's leading organisation devoted to increasing knowledge, so that the diversity of plants and fungi can be understood and used in sustainable ways for human benefit and conserved for future generations. It is a non-departmental public body and consists of two outstanding gardens, Kew Gardens, a World Heritage Site, and Wakehurst Place. These two visitor attractions are home to Kew's collections, laboratories, library and the Millennium Seed Bank. A science and plant-led organisation, the gardens crucially enable Kew to build public understanding and support for sustainability and plant conservation. It welcomed over 1.5 million visitors last year, has an income of over £40 million and involves 1,200 people, including 350 volunteers. The Trustees now seek an exceptional individual to succeed Sir Peter Crane, who leaves in 2006 after seven years to take up a Chair at the University of Chicago.

- Management responsibility for all aspects of the organisation, accountable to the Board of Trustees and to Parliament, as Accounting Officer.
- Providing leadership and vision for the organisation.
- Scientific leadership, including setting science strategy and maintaining and developing Kew as a world class centre for conservation and living plant collections.
- Ambassador for Kew, promoting its activities and international status, working with all stakeholders.
- Enhancing the outstanding values of RBG Kew while developing its public and educational mission.
- Demonstrable record of achievement in plant sciences or a related discipline.
- Senior management experience gained from a large and complex organisation.
- Accomplished communicator in public, private and political forums.
- Ability to engage with strategic fundraising initiatives and demonstrate commercial awareness.

*** commensurate with experience, including a house at Kew Gardens**

selection odgers search interim

A candidate brief containing full application details can be downloaded from our website www.odgers.com/9218 or requested by telephone or email, quoting reference AWA/9218SC. Closing date: 27th January, 2006

RBG Kew is committed to the principles of diversity in all appointments and to selection on merit with openness and transparency of process

Odgers Ray & Berndtson, 11 Hanover Square, London W1S 1JJ
t 0845 1309005 **f** 020 7529 1009 **e** nfp.response@odgers.com

www.odgers.com

UPPSALA UNIVERSITET

Chair in Physical Chemistry

at the Section of Chemistry, Faculty of Science and Technology, Uppsala University

Ref. no. UFV-PA 2005/3322

Within physical chemistry, the department presently offers postgraduate courses and research opportunities in ultrafast chemical reactions and laser spectroscopy, artificial photosynthesis, colloid and interfacial chemistry and molecular simulation. The subject is broad and new research areas are welcomed. Uppsala University is looking for a person who can either strengthen one of the existing research areas or else establish a new area of mainly experimental physical chemistry within the department. A documented theoretical ability is, however, also a qualification.

The tasks will include: Comprehensive responsibility for research and postgraduate studies in physical chemistry, teaching and advising PhD-students and undergraduate students. Research in experimental physical chemistry. Information about research and development and planning of new research projects. Administration at a divisional or higher level.

For further information about the position, please contact the Head of the Department of Physical Chemistry, Christer Elvingson, tel +46(0)18 471 3631, e-mail: Christer.Elvingson@fki.uu.se or Professor Sten Lunell, tel +46(0)18 471 3268, e-mail Sten.Lunell@kvac.uu.se.

Information about the Section of Chemistry can be found at www.chemistry.uu.se and about the Department of Physical Chemistry at www.fki.uu.se.

Further particulars including instructions for applicants can be obtained from Margareta.Sollenberg@uadm.uu.se. This information can also be found at http://www.teknat.uu.se/english/index.php.

Closing date for acceptance of applications is January 23th, 2006.

ASSISTANT/ASSOCIATE PROFESSOR IN NEUROBIOLOGY AND BIOIMAGING

The School of Life Sciences at Arizona State University is searching for a tenure-track faculty member at the Assistant or Associate Professor level to use state-of-the-art bioimaging techniques to solve current problems in neurobiology. Research in neural development or plasticity, signal transduction, and neural modulatory systems are examples of research areas in which the candidate might be working. The successful candidate will have the opportunity to contribute expertise to our W.M. Keck Bioimaging and Electron Microscopy Laboratory by participating in equipment grants and advisory panels, and to collaborate with a wide array of scientists who include the growing neurobiology research cluster in the School of Life Sciences, the Centers of Optical Biotechnology, Molecular Biophysics and High Resolution Electron Microscopy, the neural engineering group in the Ira Fulton School of Engineering, the Neurogenomics Division of the Translational Genomics Research Institute, the Barrow Neurological Institute, the T.H. Christopher Parkinson's Disease Research Center, and the Sun Health Research Institute, all in the Phoenix metropolitan area. Websites for SOLS and our bioimaging laboratories (**sols.asu.edu/klab** and **sols.asu.edu/lsem**.) provide further information.

Candidates must have a doctoral degree and a record of scholarly activity appropriate to rank Individuals who have postdoctoral research experience in a relevant discipline, and have demonstrated innovation and excellence in bioimaging research appropriate to rank, are preferred. Responsibilities of the position will include maintaining an active, extramurally funded research program, mentorship of graduate students and postdoctoral fellows, and teaching at the undergraduate and graduate levels.

To apply, candidates must submit a cover letter, curriculum vitae, and a statement of research interests and a statement of teaching experience. Additionally, for an Associate Professor position, include the name, phone number and email address for 3 references; for the Assistant Professor level, request 3 letters of recommendation to be sent to: **Chair, Bioimaging/Neurobiology Search Committee, School of Life Sciences, P.O. Box 874501, Arizona State University, Tempe, AZ 85287-4501**. Letters of reference, ***but not application materials***, may be submitted by email to **nicole.barr@asu.edu**. Application deadline is January 20, 2006; if not filled, applications will be reviewed weekly thereafter until the search is closed. See **http://sols.asu.edu/jobs/index.php** for more information. A background check is required for employment.

Arizona State University is an Affirmative Action-Equal Opportunity Employer.

Department Head
Department of Biochemistry and Molecular Biology
Franklin College of Arts and Sciences
University of Georgia

The University of Georgia is seeking an established scientist with creative vision to lead the Department of Biochemistry & Molecular Biology [http://www.bmb.uga.edu/home/]. With over 30 faculty members, the internationally recognized department has research strengths in several areas, including glycobiology, structural biology, bioinformatics, microbial genomics, enzyme mechanisms, and molecular/cellular biology. This search is coincident with a dramatic expansion of research programs in interdisciplinary biomedical and health sciences at UGA, the establishment of a new College of Public Health, the development of state-of-the-art biohazard containment facilities, and increasing emphasis on quantitative approaches to biological and medical problems [see **http://www.ovpr.uga.edu/facultypositions/**].

Candidates should have an outstanding record of academic accomplishments and funding, as well as proven leadership and administrative skills. Send applications and nominations electronically to **Dr. Wyatt W. Anderson, Chair of the Search Committee, chairheadsearch@bmb.uga.edu**. Applicants should submit a cover letter summarizing their qualifications and vision, together with complete curriculum vitae. For full consideration, applications must be received by **January 5, 2006**.

The University of Georgia is an Equal Opportunity/Affirmative Action Employer.

Director of Center for Computational Neuroscience

Stony Brook University seeks an outstanding scientific leader to serve as founding Director of a new Center for Computational Neuroscience. The successful candidate will have expertise in computational neuroscience and demonstrated leadership ability to build upon traditional disciplines in neurobiology, psychology, other biomedical sciences, physical sciences, electrical engineering, computer science, and mathematics to develop integrative, research, and training programs in computational neurosciences. The Center Director will have the resources to recruit new faculty to Stony Brook University in coordination with Neurobiology and other relevant departments in the College of Arts and Science, College of Engineering and Applied Sciences, School of Medicine, and in collaboration with the neighboring Brookhaven National and Cold Spring Harbor Laboratories. Substantial resources to establish this Center of Excellence have been provided by New York State and Stony Brook University for faculty recruitment and infrastructure development.

Required: M.D., Ph.D., or equivalent degree; the academic rank of Associate or Full Professor; extramural funding at a national/international level; publications in peer-reviewed journals, book chapters, and reviews; and symposium participation at the national/international level. The candidate will also have a proven record of success in graduate student and/or post-doc training.

The review of applications will begin January 1, 2006, and will continue until the position is filled.

Applicants should forward a curriculum vitae to:
Computational Neuroscience Director Search Committee
c/o Maria Doelger, 407 Administration Building
Stony Brook University, SUNY, Stony Brook, NY 11794-1401
or e-mail *CompNeuroSearch@notes.cc.sunysb.edu*

AA/EOE. Visit *www.stonybrook.edu/cjo* for complete job description and other employment opportunities.

STONY BROOK

Biology Faculty

The Department of Biology at Rensselaer Polytechnic Institute seeks candidates in any area of basic biomedical research for tenure track faculty positions at all academic levels. We are particularly interested in candidates in the areas of genomics, proteomics, and bioinformatics as part of a campus-wide initiative in computational biology and bioinformatics. Rensselaer has recently opened a 218,000 sq. ft. Center for Biotechnology and Interdisciplinary Studies with approximately 60 faculty laboratories and state of the art core facilities. Significant funding is available for startup packages. Review of applications will begin January 15, 2006, but searches will continue until the position is filled. Please send a curriculum vitae, a statement of research interests up to three pages, and a minimum of three letters of reference to:

Robert H. Parsons, Chair Biology Search Committee

Biology Department, Rm. 1W14 SC

Rensselaer Polytechnic Institute

110 8th Street, Troy, New York 12180-3590

We welcome responses from those persons who will bring diverse intellectual, geographical, gender and ethnic perspectives to Rensselaer's work and campus communities.

Rensselaer Polytechnic Institute is an Affirmative Action/Equal Opportunity Employer.

Rensselaer

LOUISIANA STATE UNIVERSITY

College of Basic Sciences and
The School of the Coast and Environment
Baton Rouge, Louisiana 70803

The College of Basic Sciences and The School of the Coast and Environment invite applications for eleven (11) tenure-track positions beginning in August 2006. Applicants should have a Ph.D. in an appropriate field. Successful candidates will be expected to develop a strong research program and contribute to graduate and undergraduate education.

The positions in the **College of Basic Sciences** are distributed among five academic departments. While we anticipate hiring at the Assistant Professor level, candidates at higher ranks are also encouraged to apply.

The **Department of Biological Sciences** is seeking a faculty member in the area of Microbial Ecology/Evolution **(Log #0464)**.

The **Departments of Biological Sciences and Chemistry** jointly invite applications for a tenure-track position in the area of Toxicogenomics **(Log #0458)**.

The **Department of Chemistry** invites applications for three tenure-track positions in the areas of (1) NMR Spectroscopy/ **Log #0423**, (2) Physical Chemistry/**Log #0421**, and (3) Macromolecular or Organic Chemistry/**Log #0422**.

The **Department of Computer Science** invites applications for a tenure-track position, which is open to outstanding candidates in all areas of Computer Science **(Log #0465)**.

The **Department of Geology and Geophysics** invites applications and nominations for the Charles T. McCord Chair in Petroleum Geology **(Log #0508)**.

The **Department of Physics and Astronomy** invites applications for an Assistant Professor in the area of Medical Imaging Physics **(Log #0463)**.

Further information for Basic Sciences positions is available at **science.lsu.edu/employment.htm**.

The School of the Coast and Environment has three positions available in the **Department of Oceanography and Coastal Sciences:**

Biological Oceanographer (Assistant Professor/Log #0551) with expertise in one or more of the following areas: phytoplankton ecology, microbial ecology, coastal and estuarine primary productivity, noxious and toxic algal species, or microbial processes.

Coastal Marine Geologist (Assistant/Associate Professor/ Log #0552) with expertise in the general areas of shelf, coastal, estuarine, and fluvial processes.

Physical Oceanographer (Assistant Professor/Log #0553) with expertise in the general area of continental shelf or larger scale ocean circulation and demonstrated research interests (observational and/or modeling) in one or more of the following areas: circulation and transport processes, mixing processes, ocean/atmosphere interactions, gravity wave dynamics (including internal waves), and climate change.

Further information for the School of the Coast and Environment positions is available at **www.ocean.lsu.edu**.

For more information and complete qualifications, see our website at **http://www.lsu.edu/lsucareers**.

LSU IS AN EQUAL OPPORTUNITY/EQUAL ACCESS EMPLOYER.

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

Bereich Humanmedizin

Universitätsklinikum – Medizinische Fakultät

The Bereich Humanmedizin (University Hospital and Faculty of Medicine) of the Georg-August-Universität Göttingen seeks to fill a

PROFESSORSHIP (W2-PROFESSUR, TENURE TRACK) IN MOLECULAR MICROSCOPY

which will be installed at the Department of Neuro- and Sensory Physiology as part of the joint initiative with the newly established DFG-Research Centre for the *Molecular Physiology of the Brain (CMPB).*

We are looking for candidates with profound experience in the optical investigation of molecular processes in single neurons, aiming at physiological systems analysis. The goal is the quantification of biochemical networks in time and space and their correlation with cellular neurophysiology. Therefore, we expect expertise in quantitative *in vitro* and *in vivo* fluorescence detection techniques. The applicant should have a broad experience in the application of biophotonic techniques; in the design and improvement of innovative optical bioassays for specific neuronal functions; and in the implementation and development of optical instrumentation/analysis solutions.
In teaching, the position is dedicated to the whole field of Physiology, as well as to the curriculum in the MSc-PhD graduate program in Neurosciences. The CMPB Centre and the department offer a unique environment for interdisciplinary research in the Neurosciences and Biophysics. An excellent infrastructure is available at the department.

The formal requirements for the recruitment follow § 25 NHH. Details can be given upon inquiry.

Handicapped candidates are given priority, if equally qualified.

The University of Göttingen is determined to increase the percentage of female professors. Therefore, we strongly encourage qualified female scientists to apply. A part-time appointment can eventually be given.

Applications with the standard documents (CV, index of publications, presentation of teaching record, diplomas) should be sent in by 4 weeks to

Dekan der Medizinischen Fakultät des Bereichs Humanmedizin der Georg-August-Universität, Robert-Koch-Str. 42, 37075 Göttingen

Please fill in as well the following form:
http://www.humanmedizin-goettingen.de/orga/doc/curriculum_vitae-kurzbewerbungsbogen.doc

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Assistant Professor (Tenure Track) of Experimental Geochemistry

The Department of Earth Sciences is seeking an experimentalist with a strong theoretical background to lead investigations on the structure and properties of natural materials with innovative applications to Earth science problems.

He or she will develop an experimental program including in-situ observations (i. e. synchrotron and other microbeam methods) of the behaviour of natural materials at high pressures and high temperatures, as well as computational chemistry applied to geochemical problems. Potential applications might include melts and fluids in planetary interiors and surfaces, the transport properties of geologically and environmentally relevant fluids, and the abiotic origin of organic molecules in volcanic and planetary environments. The new professor will join the Institute of Mineralogy and Petrology and initiate cooperative research/teaching programs with other groups at ETH and related institutions.

The successful candidate will have a record of accomplishments in one or more of the fields of physical and chemical properties of fluids and gases, melts, and/or minerals at high pressures and high temperatures.

The new professor and his or her staff will offer introductory and advanced courses covering physical chemistry aspects of the Earth's subsurface and interior. Courses at Master level may be taught in English.

This assistant professorship has been established to promote the careers of younger scientists. The initial appointment is for four years with the possibility of renewal for an additional two-year period and promotion to a permanent position.

Applicants should submit a detailed résumé, publication list, statement of research interests, and the names of three potential referees to **the President of ETH Zurich, Professor E. Hafen, ETH Zentrum, CH-8092 Zurich, Switzerland no later than January 31, 2006.** With a view towards increasing the proportion of female professors, ETH Zurich specifically encourages female candidates to apply.

PATHOLOGY AND LABORATORY MEDICINE

UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE

INSTITUTE ON AGING/BIOINFORMATICS

ASSISTANT PROFESSOR

The Department of Pathology and Laboratory Medicine and the Institute on Aging at the University of Pennsylvania's School of Medicine seeks candidates for an Assistant Professor position in the tenure track. The successful applicant will have experience in the field of Neurodegerative Disease with a focus on Bioinformatics related to normal aging and aging related neurodegenerative diseases. Applicants must have a Ph.D. or M.D./Ph.D. degree and have demonstrated excellent qualifications in Education and Research.

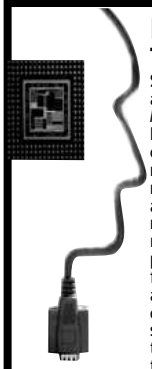
This faculty appointment would include membership in the Institute on Aging. The ideal candidate should have demonstrable abilities and background to develop an independent research program in bioinformatics of aging and aging related neurodegenerative diseases. In particular, candidates are expected to possess bioinformatics expertise in the field of aging research. Teaching and mentoring of medical students, pathology residents, biomedical graduate students, and fellows will be an integral component of the position. All applications should be received by February 1, 2006.

The University of Pennsylvania is an equal opportunity, affirmative action employer. Women and minority candidates are strongly encouraged to apply.

Please submit curriculum vitae, a brief statement of research interests, and three reference letters to:

Virginia M.-Y. Lee, Ph.D., Search Committee Chair
C/O Gayle Joseph
University of Pennsylvania School of Medicine
Institute on Aging
3 Maloney-HUP, 3600 Spruce St., Phila., PA 19104-4283
viale@mail.med.upenn.edu

http://www.uphs.upenn.edu/path/JobOpps.html



Los Alamos National Laboratory
Title: Group Leader, T-12

Summary: Los Alamos National Laboratory is accepting applications for the position of Theoretical Chemistry and Molecular Physics (T-12) Group Leader. The T-12 Group Leader provides scientific leadership, project management, capability development, and line management. The members of the T-12 group seek to understand the behavior of materials by describing how basic forces operating at the atomic and molecular level manifest themselves in the properties of matter at more macroscopic levels. Current activities include research in gas phase and condensed phase phenomena. Research projects include the development and application of techniques for calculating the electronic properties of molecules and solids, atomistic simulations of materials, the dynamics and kinetics of chemical reactions, molecular modeling of catalysts, and the study of solute-solvent interactions. Particular applications of this research are to the properties of actinide materials and transition metals, to the properties of polymers, biological solvation processes, and fuel cell technologies. Work in the group supports applied missions of the Laboratory, including the Advanced Simulation and Computing (ASC) program, Threat Reduction (TR) programs, and the Department of Energy (DoE) Basic Energy Sciences programs. The Group Leader, with the help of the Deputy Group Leader, will develop and manage the Group's human, financial, computing, and other resources; new programs and funding. The Group Leader will also maintain an active research effort, at the half to three-fourths level.

Required Skills: Demonstrated knowledge and research accomplishments in one or more of T-12's technical research focus areas. Ability to provide scientific and project leadership, project management skills, and fiscally responsible business practices. Demonstrated ability to function effectively in an environment of rapidly changing priorities. Excellent communication skills. Attract and establish research programs from sponsors comparable to those such as the DoE, Laboratory LDRD, industrial partners, and other agencies. Ability to obtain a DoE Q clearance, which normally requires U.S. citizenship.
Ph.D. in Chemistry, Physics, Material Sciences, or the equivalent combination of education and relevant experience.
For a complete job description and application information, visit www.lanl.gov/jobs and search for job# 211737.

Los Alamos National Laboratory is operated by the University of California for the National Nuclear Security Administration of the Department of Energy. AA/EOE

Put your brain to good use.

Los Alamos NATIONAL LABORATORY

www.lanl.gov/jobs

10YR ANNIVERSARY

جامعة كارنيجي ميلون قطر
Carnegie Mellon QATAR CAMPUS

مؤسسة قطر Qatar Foundation

Human-Computer Interaction Visiting Faculty Position in the School of Computer Science

Carnegie Mellon University established a branch campus in Qatar in the fall of 2004. We are offering a BS degree in Computer Science to an international student body. The university invites applications for a visiting faculty position to begin as early as January 2006.

We are seeking a faculty member in the area of Learning Science and Technology with research experience ideally in designing, implementing, deploying, and evaluating educational technology in school or college settings. An ability to teach courses in human-computer interaction, artificial intelligence, cognitive psychology, or related areas is also desired. The position will involve research in collaboration with the Pittsburgh Science of Learning Center and faculty at the Human-Computer Interaction Institute at Carnegie Mellon in Pittsburgh. The position offers competitive salaries, overseas assignments, travel and housing allowances and other benefits packages, as well as attractive research support.

Interested candidates should send their resume, statement of teaching interest and research, and names of three references to: **Faculty Hiring Committee, c/o Ruth Gaus, Qatar Office SMC 1070, 5032 Forbes Avenue, Pittsburgh, PA 15289; Ruth.Gaus@cs.cmu.edu; Fax 412-253-0924.**

- For more information on the Pittsburgh Science of Learning Center, see **http://learnlab.org**.
- For more information on the Human-Computer Interaction Institute, see **http://www.hcii.cs.cmu.edu**.
- For more information on the BS in CS program, see **http://www.csd.cs.cmu.edu/education/bscs/index.html**.
- For more information on the Carnegie Mellon Qatar Campus, see **http://www.qatar.cmu.edu/.**
- Information on Qatar is available at: **http://www.experienceqatar.com/**.

The Ohio State University

Professor and Chair

Department of Biomedical Engineering

The Ohio State University (OSU) seeks candidates for the position of Professor and Chair of Biomedical Engineering. The Biomedical Engineering (BME) Center with a Graduate Program, established in 1971, was established as a department within the College of Engineering on November 4, 2005. Situated within one of the largest comprehensive, land-grant universities, BME enjoys extensive collaborations with faculty in engineering and health science colleges including medicine, dentistry, veterinary medicine and biological sciences and engineering. BME currently has 15 core faculty members, 59 participating members, and 5 support staff members. BME administers a strong graduate program (MS & PhD), participates with a MD/PhD program, and offers an undergraduate minor curriculum. An undergraduate major curriculum is in the planning stages. Research in the BME center is focused in three interrelated areas: Tissue engineering/biomaterial, biomedical micro- & nano-technology, and bio imaging. Related, specialized research facilities and equipment are shared campus-wide. For more details see: www.bme.ohio-state.edu.

The successful applicant **will present leadership skills and an extensive record of accomplishment in research and scholarship: He or she will lead a research program fitting the departmental focus areas, demonstrate biomedical teaching experience, and have a record of extensive extramural funding.** In addition, the candidate will develop and implement a strong vision for the future of the department with the objective of achieving a high national ranking.

OSU is committed to excellence in undergraduate and graduate education, and to diversity. Applications should include a curriculum vita, a statement of the candidate's vision for the future of biomedical engineering research and education, a self-assessment of leadership qualities and style, and the names of five professional references. Review of applications will begin immediately and continue until the position is filled.

Please send applications to: **BME Chair Search Committee, c/o Kirsten Gibbons, 270 Bevis Hall, 1080 Carmack Rd., Columbus, Ohio 43210, Email: gibbons.40@osu.edu**

Search Committee Chairs
Sanford Barsky, Professor & Chair
Department of Pathology, College of Medicine
Stuart L. Cooper, Professor & Chair
Department of Chemical and Biomolecular Engineering
College of Engineering

THE OHIO STATE UNIVERSITY

To build a diverse workforce Ohio State encourages applications from individuals with disabilities, minorities, veterans, and women. EEO/AA employer.

UC DAVIS
MUSCLE PHYSIOLOGIST

The Section of Neurobiology, Physiology and Behavior, in the College of Biological Sciences, University of California, Davis, invites applications for a faculty position in Physiology at the assistant professor level. Applicants specializing in skeletal or cardiac muscle physiology are especially encouraged to apply. Areas of significant interest include, but are not limited to, exercise physiology, muscle development and regeneration, metabolic control, calcium signaling, and biomechanics. Successful applicants will be expected to establish a vigorous research program supported by extramural funding, and contribute to the teaching mission of the Section, including the Exercise Biology major. The Section has been steadily expanding since its inception in 1993 to include 32 ladder rank faculty who conduct research encompassing a general theme of integrative biology, ranging from muscle physiology and biomechanics, molecular endocrinology, environmental physiology, cell physiology, aging, molecular, cellular, and developmental neurobiology, systems neuroscience, and animal behavior. In addition, UC Davis has one of the largest concentrations of life scientists in the world, with vibrant units across campus that would provide the successful candidate with a wide range of collaborative interactions. These units include the Department of Physiology and Membrane Biology in the Medical School, the Exercise Biology Program (now fully integrated into the Section), the UC Davis Genome Center, the Mouse Biology Program, the Clinical Nutrition Research Unit, the Molecular, Cellular, and Integrative Physiology and Exercise Science Graduate Groups, the Center for Neuroscience, the Department of Biomedical Engineering, and other physiology-related departments of the Schools of Medicine and Veterinary Medicine and College of Agriculture and Environmental Sciences.

Candidates must possess a Ph.D. or M.D. degree with significant post-doctoral experience. Applicants should send a letter describing their research plan and teaching interests, a curriculum vitae, copies of representative publications, and the names of at least five persons from whom references can be obtained to: **Chair, Muscle Physiology Search Committee, Section of Neurobiology, Physiology, and Behavior, One Shields Avenue, University of California, Davis, CA, 95616-8519**. All materials must be received by **February 1, 2006**, to be assured full consideration. For more information on the position and UC Davis in general, please visit the following web site: **www.npb.ucdavis.edu/facultypositions/**.

The University of California is an Affirmative Action/Equal Opportunity Employer.

The University of Arizona
Tucson, Arizona

Department of Plant Sciences
Department Head and Professor

Position Description: The College of Agriculture and Life Sciences invites applications and nominations for the position of Head of the Department of Plant Sciences and Professor. The Department of Plant Sciences, in addition to serving the agricultural industry of the state of Arizona, has extensive collaborative interactions with faculty in the biological sciences across the University. The department consists of 40 faculty members who reside on the main campus in Tucson and at several off-campus experimental stations located elsewhere in the state. The department is also home to the University of Arizona Campus Herbarium, the largest herbarium in the southwest. Strong innovative, interdisciplinary leadership in an academic environment is being sought to lead the Department in the areas of extension, research, and teaching. The candidate should have established scholarly credentials in an area that could be considered a part of a contemporary inter-disciplinary department of plant sciences, including agronomy, biochemistry, botany, controlled environment agriculture, genetics, genomics, horticulture, molecular and cellular biology, plant-microbe interactions, plant-insect interactions, plant physiology or development. The successful candidate will be encouraged to maintain an extension, research, or teaching program. The department is seeking an individual who is able to work with diverse students and colleagues, and who has experience with a variety of teaching methods and curricular perspectives.

Qualifications: A Ph.D. is required, preferably in one of the above fields or areas of study. Strong evidence of managerial and leadership abilities, the capacity to articulate a vision for the Department, and a strong appreciation for diversity and for the land-grant mission are essential.

Salary: The salary will depend upon qualifications and experience.

Application Procedure: Applicants should submit a current curriculum vitae, the names, addresses (including email), and telephone numbers of six references, and a statement of qualifications and goals to: **Dr. Jeffrey C. Silvertooth, Plant Sciences Search Committee, Department of Plant Sciences, University of Arizona, Forbes Building, Room 303, PO Box 210036, Tucson, AZ 85721-0036. Phone: (520) 621-1977 Fax: (520) 621-7186. pltsci@cals.arizona.edu**. Position # **34015**.

The committee will begin considering applicants on **1 February 2006** but will accept applications until suitable candidates for interviews have been identified. This appointment will be available 1 July 2006.

The University of Arizona is an Equal Employment Opportunity/Affirmation Action Employer. Women and minorities are especially encouraged to apply.

POSITIONS OPEN

EVOLUTIONARY ECOLOGIST

The Department of Biological Sciences at Binghamton University, one of the four doctoral granting State University of New York University Centers, invites applications for a new position at the Assistant Professor level in evolutionary ecology. We seek someone who can establish a strong, extramurally funded empirical research program informed by evolutionary theory. Research that integrates evolutionary ecology with other disciplines (e.g., ecosystem ecology, genetics) will receive special attention. This position is designed to contribute to cross-departmental integration through EvoS **(website: http://bingweb.binghamton.edu/~evos/),** our campus-wide evolutionary studies program. Applicants should submit curriculum vitae, statement of research and teaching interests, sample of reprints, and have three letters of reference sent to:

Dr. David Sloan Wilson
Chair, Evolutionary Ecology Search Committee
Department of Biological Sciences
Binghamton University
State University of New York
Binghamton, New York 13902-6000.

Review of applications will begin on January 1, 2006, and will continue until the position is filled. *Binghamton University is an Equal Opportunity/Affirmative Action Employer.*

ANIMAL ECOLOGIST

We seek a broadly-trained Animal Ecologist for a tenure-track assistant professorship who uses individual-based physiological or behavioral research approaches to understand population, community, or evolutionary processes. The successful candidate will develop an externally-funded research program and contribute to both undergraduate and graduate education in biological sciences and the Conservation and Environmental Sciences Program. Postdoctoral experience is expected. Submit a letter of application, curriculum vitae, statements of research and teaching interests and the names, addresses, and e-mail contact of three references in PDF format to **e-mail: ecosearch@uwm.edu,** or by post to:

Ecologist Search
Department of Biological Sciences
University of Wisconsin, Milwaukee
P.O. Box 413
Milwaukee, WI, 53201

Additional information is at **website: http://www.uwm.edu/Dept/Biology.** Review of applications will begin on January 23, 2006, and will continue until the position is filled. *University of Wisconsin, Madison is an Affirmative Action/Equal Opportunity Employer.*

FACULTY POSITION
University of Wisconsin, Madison

Assistant Professor tenure-track position in exercise physiology available starting August 28, 2006. Earned doctorate is required and postdoctoral research experience is preferred. Area of research interest is open within the broad confines of biological aspects of exercise. Information about the Department is available at **website: http://www.education.wisc.edu/kinesiology/.** Applicants should send a letter of application, curriculum vitae, statement of research interests, copies of up to three published articles in refereed journals, and names, mailing addresses, e-mail addresses, and telephone numbers of three references to: **Professor Gary Diffee, University of Wisconsin, Department of Kinesiology, 2000 Observatory Drive, Madison, WI 53706. E-mail: diffee@education.wisc.edu.** (PVL 52124) To ensure full consideration, applications should be received by February 1, 2006. Unless confidentiality is requested in writing, information regarding the applicants must be released upon request. Finalists cannot be guaranteed confidentiality.

University of Wisconsin, Madison is an Equal Opportunity/Affirmative Action Employer. We promote excellence through diversity and encourage all qualified individuals to apply.

POSITIONS OPEN

The Cancer Center

UNIVERSITY OF MINNESOTA

ASSISTANT PROFESSOR

The University of Minnesota Cancer Center Breast Cancer Program invites applications for a tenure-track Assistant Professor. Candidates are expected to have a Ph.D. or relevant doctoral degree. The successful candidate will have completed a postdoctoral fellowship and have an interest in targeted therapies in breast cancer. Experience in developing pre-clinical models of targeted therapies, identifying predictive biomarkers, and utilizing in vivo imaging modalities in conjunction with targeted therapies are required. There are numerous opportunities for collaboration within the Cancer Center. In addition to developing and maintaining their own vigorous research program, the successful candidate will also participate in the development of an interdisciplinary translational breast cancer research program. The Cancer Center also supports substantial Shared Resources and the applicant will have access to these facilities. Academic appointment will be made in an appropriate academic unit within the University of Minnesota's Academic Health Center. Interested applicants should send their curriculum vitae, a summary of research interests, and three letters of recommendation to: **Dr. Douglas Yee, c/o Human Resources, MMC 806, 420 Delaware Street S.E., Minneapolis, MN 55455. E-mail: cchr@umn.edu.** Application deadline is January 31, 2006. *The University of Minnesota is an Equal Opportunity Educator and Employer.*

THE CLEVELAND CLINIC FOUNDATION
Lerner Research Institute

The Pathobiology Department of The Cleveland Clinic Lerner Research Institute invites applications for new faculty at the **ASSISTANT, ASSOCIATE OR FULL PROFESSOR** position in integrative pharmacology/physiology.

This position is an exceptional opportunity to translate laboratory discoveries to the patient through the basic and clinical resources of The Cleveland Clinic. The successful candidate will possess either a M.D. or Ph.D. in pharmacology, physiology or related field, with a strong track record of accomplishments. Emphasis on integrated approaches to disease-related research or experience in pharmaceutical or biotechnology industry is desirable. Outstanding facilities and generous startup funds are available. Interactions with excellent clinical programs are readily available and provide the potential to develop multi-disciplinary research programs. The Lerner Research Institute has a strong tradition of collaborative research and well-developed training programs for postdoctoral fellows and medical students. Candidates should submit curriculum vitae, summary of research plans, and three references to:

Serpil C. Erzurum, M.D.
Chair, Pathobiology
The Cleveland Clinic Foundation
9500 Euclid Avenue/NB40
Cleveland, Ohio 44195.

ASSOCIATE RESEARCH SCIENTIST

Ph.D. or preferably M.D./Ph.D. with at least four years of experience to run basic science laboratory, to design and perform clinical and in vitro studies in the field of cancer biology. Experienced in mammalian cell culture, isolation of DNA and RNA, PCR and RT-PCR, DNA constructs, preparation of cell lysates, immunomagnetic protein purification, Western blot, zymography, enzyme-linked immunosorbent assay, immunohistochemistry, immunofluorescent staining, microscopy/confocal microscopy, and flow cytometry.

Fax resumes to **fax: 212-305-9474, Attn: Debra Keller.** *Columbia University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

INVERTEBRATE ZOOLOGIST

Tenure-track position, Assistant Professor level, beginning August 2006. Strong commitment to excellence in teaching and research essential. Teaching responsibilities include introductory majors course and upper-level courses in entomology and invertebrate zoology. An active research program involving graduate and undergraduate students, and pursuit of extramural funding expected. Successful candidate eligible to compete with other junior faculty for a three-year appointment as the newly established Jess Fisher Endowed Chair in the Biological and Physical Sciences. Holder of Chair will receive additional funds to support her/his research program. Ph.D. required, postdoctoral experience preferred. Applications should include curriculum vitae, photocopies of transcripts from all institutions attended, one-page statements of teaching philosophy and research interests, and three letters of reference. Review of applications begins January 15, 2006, and continues until position is filled. Submit applications to: **Invertebrate Zoology Search Committee Chair, Department of Biological Sciences, Towson University, Towson, MD 21252.** Additional information available at **website: http://wwwnew.towson.edu/biology/.** *Towson University is an Equal Opportunity, Affirmative Action Employer and has a strong institutional commitment to diversity. Women, minorities, persons with disabilities, and veterans are encouraged to apply.*

LIFE SCIENCES TEACHING POSITION
University of Houston

The Honors College and the Department of Biology and Biochemistry at the University of Houston (UH) invite applications for an instructional Assistant Professor to contribute to the teaching mission of these units. This position is a twelve-month, non-tenure-track appointment, with the possibility of annual renewal. The successful applicant will teach honors sections of courses in introductory biology and genetics, and will coordinate special programs for honors students in the natural sciences. The position requires an earned doctorate in any appropriate area of the life sciences, broad knowledge of biology and biochemistry, prior teaching experience at the post-secondary level, and a commitment to high quality undergraduate teaching. Review of applications will begin February 1, 2006. Start date: June 1, 2006, preferred. Please submit curriculum vitae, a statement outlining teaching experience and philosophy, and three letters of recommendation to: **Honors Biology Search Committee, The Honors College, 212 M.D. Anderson Library, University of Houston, Houston, TX 77204-2001.** *UH is an Equal Opportunity/Affirmative Action Employer. Minorities, women, veterans, and persons with disabilities are encouraged to apply.*

Wheaton College, Wheaton, Illinois, seeks candidates for an **ASSISTANT OR ASSOCIATE PROFESSOR** tenure-track position in physics to begin in August, 2006. Field of expertise is open within the general area of experimental physics; special consideration will be given to abilities in leadership, teaching, and promise for sustained scholarship in an undergraduate liberal arts college. The ideal candidate will be able to serve as departmental Chair. Two national laboratories are within twenty miles of campus.

Review of applications will begin December 15, 2005, and continue until the position is filled. Applicants should send curriculum vitae and a description of the applicant's teaching philosophy and research interests to: **Dr. Dorothy F. Chappell, Dean of Natural and Social Sciences, Wheaton College, Wheaton, IL 60187.** Application materials will be sent to eligible candidates.

Wheaton College is an evangelical protestant Christian liberal arts college whose faculty members affirm a Statement of Faith and the moral and lifestyle expectations of our Community Covenant. The College complies with federal and state guidelines of nondiscrimination in employment; women and minorities are encouraged to apply.

The **Department of Anthropology and the College of Medicine at the University of Illinois at Urbana-Champaign** seek to hire an anthropologist for a **full-time tenure-track Assistant Professor** position beginning August 16, 2006. We are interested in candidates with a research program in functional or developmental morphology, medical anthropology or human biology, human or primate paleontology. Consideration also will be given to all candidates with research experience in other areas of anthropology that complement existing strengths in the Department of Anthropology. Candidates must be willing to assume responsibility for lecture and laboratory instruction in human gross anatomy in the College of Medicine, and have demonstrated excellence as a human gross anatomy instructor. Scholarly excellence is our primary criterion. A Ph.D. degree in a relevant biological, anthropological, or medical discipline is required, and applications from medical scholars (MD/PhD) are strongly encouraged. Salary will be commensurate with experience, and benefits will be consistent with full-time University employment.

Please send a letter of application, vitae, samples of publications, a statement detailing research interests and plans, teaching experience and the names and addresses of three referees to: **Paul A. Garber, Head, Department of Anthropology, 109 Davenport Hall, 607 S. Mathews Avenue, Urbana, Illinois 61801; 217-333-3616**. Full consideration will be given to applications received by **February 15, 2006**. Position **10241**.

The University of Illinois is an Affirmative Action/Equal Opportunity Employer.

UC DAVIS ENDOWED CHAIR IN PHYSIOLOGY

The Section of Neurobiology, Physiology and Behavior, in the College of Biological Sciences, University of California, Davis, invites applications for an Endowed Chair in Physiology at the full professor level, to begin July 1, 2006. Outstanding applicants specializing in any area of physiology consistent with the broad goals of the Section will be considered. The Section has made a significant commitment to the growth of physiology including several junior faculty positions in addition to the appointment of the Endowed Chair. The Section has been steadily expanding since its inception in 1993 to include 32 ladder rank faculty who conduct research encompassing a general theme of integrative biology, ranging from muscle physiology and biomechanics, molecular endocrinology, environmental physiology, cell physiology, aging, molecular, cellular, and developmental neurobiology, systems neuroscience, and animal behavior. In addition, UC Davis has one of the largest concentrations of life scientists in the world, with vibrant units across campus that would provide the successful candidate with a wide range of collaborative interactions. These units include the Department of Physiology and Membrane Biology in the Medical School, the Exercise Biology Program (now fully integrated into the Section), the UC Davis Genome Center, the Mouse Biology Program, the Clinical Nutrition Research Unit, the Molecular, Cellular, and Integrative Physiology Graduate Group, the Center for Neuroscience, the Department of Biomedical Engineering, and other physiology-related departments of the Schools of Medicine and Veterinary Medicine and the College of Agriculture and Environmental Sciences. For more information on the position and UC Davis in general, please visit the following web site: **www.npb.ucdavis.edu/facultypositions**.

Successful applicants will be expected to maintain a vigorous research program with continued extramural funding, and contribute to the teaching mission of the Section. Candidates must possess a Ph.D. or M.D. degree with significant experience as an established independent investigator and academic leadership. Applicants should send a letter describing their research interests, curriculum vitae, copies of representative publications, and the names of at least five persons from whom references can be obtained to: **Chair, Endowed Chair in Physiology Search Committee, Section of Neurobiology, Physiology, and Behavior, One Shields Avenue, University of California, Davis, CA, 95616-8619**. Review of applications will commence **February 1, 2006**, and the search will continue until the position has been filled.

The University of California is an Affirmative Action/Equal Opportunity Employer.

ALBERT EINSTEIN COLLEGE OF MEDICINE CANCER CENTER POSTDOCTORAL OPPORTUNITIES

Applicants are invited to apply for postdoctoral positions in laboratories engaged in interdisciplinary, collaborative research at the Albert Einstein Cancer Center, a major NCI-designated research institute with a broad spectrum of programs and core laboratory facilities. Research training areas encompass:

- Epigenetics, chromatin, transcriptional regulation, and tumor suppressors
- Thermodynamics/kinetics of DNA-protein interactions and RNA folding
- Leukemia, Lymphoma, Myelodysplastic Syndrome
- Human embryonic stem cells, cell cycle and differentiation controls
- Mismatch repair and AID-induced mutations in B cell lymphomagenesis
- Growth factors, steroid hormones, signaling and cancer
- The role of macrophages in breast cancer progression
- Glycan functions in tumorigenesis, metastasis and Notch signaling
- Transcriptional regulation of drug metabolism
- Molecular cloning and characterization of novel facilitative anticancer drug transporters
- Ribosomal synthesis-relation to cell growth, division and apoptosis
- Regulatory mechanisms in lower organisms

To learn more about specific research projects and their faculty preceptors go to the Albert Einstein Cancer Center Webpage at: www.aecom.yu.edu/cancer. Click on postdoctoral training.

The Albert Einstein College of Medicine is located in a residential area of the Northeast Bronx, in close proximity to City Island and Westchester County with easy access to Manhattan. Apply through the Cancer Center Web Page or write to: **Dr. Richard Seither, Albert Einstein Cancer Center, Jack and Pearl Resnick Campus, Chanin Two, 1300 Morris Park Avenue, Bronx, New York 10461.** EOE

ALBERT EINSTEIN COLLEGE OF MEDICINE
Advancing science, building careers

9 Senior Research Positions at Swedish Universities

The Swedish Research Council announces nine Senior Research Positions within natural and engineering sciences. The positions are intended for scientists who have obtained a Ph.D., where the date of exam, with a few exceptions, is not older than ten years prior to the end of the application period. The intention is that the major part of the time should be spent on research. The positions are financed for a maximum of six years.

There is one position each within the following areas:

- Speciation
- Eukaryotic Molecular Biology
- Physics at the Large Hadron Collider Facility – CERN
- Green Chemistry including Organo Catalysis
- Fast Collision Dynamics of Complex Atomic and Molecular Systems
- Molecules in Solids, Liquid Crystals or Aggregates Studied by Nuclear Magnetic Resonance Spectroscopy
- Process Technology for New Nano and Micro Structured Materials
- Reconstructing Climate over the Last Millenia
- Engineering Mechanics

The proposal must be approved by a Swedish host university or a Swedish host institution engaged in research.

Apply at www.vr.se no later than February 14, 2006
Application form and instructions can be found at www.vr.se/english.

Vetenskapsrådet
Swedish Research Council

POSITIONS OPEN

The Division of Biological Sciences in the College of Science at Marshall University invites nominations and applications for the position of **DIVISION HEAD.** The primary responsibility of the Division Head is to chair the Department of Biological Sciences. This is a twelve-month appointment with an anticipated start date of July 1, 2006. Salary and rank will be commensurate with experience.

For consideration, a candidate must possess a doctorate in the biological sciences and must demonstrate (1) the appropriate administrative skills to lead a department whose research interests are centered in environmental/organismal biology and cellular/molecular biology; (2) a distinguished record of funded research and peer-reviewed publications; and (3) a commitment to diversity in students, staff, and faculty.

The successful candidate will promote quality educational and research opportunities for undergraduate and graduate students, will support faculty development and research, and will build upon current interdisciplinary teaching and research initiatives.

Formal review of applications will begin on February 1, 2006, and continue until the position is filled. An applicant must submit (1) a curriculum vitae; (2) a letter detailing qualifications and leadership experience; (3) a statement of administrative philosophy; and (4) a vision for biological sciences for the next ten years. Please provide contact information (address, telephone number, and e-mail) of three professional references. Send materials to:

Division Head Search Committee
Division of Biological Sciences
Marshall University
One John Marshall Drive
Huntington WV 25755

or in the form of a PDF document to **e-mail: BiologyHeadSearch@marshall.edu.**

Information about Marshall University and Huntington, West Virginia can be found at **websites: http://www.marshall.edu** and **www.hadco.org.** *Marshall University is an Affirmative Action/Equal Employment Opportunity Employer and encourages applications from women, minorities and persons with disabilities.*

DEVELOPMENTAL BIOLOGIST

Illinois Wesleyan University seeks an Assistant Professor of Developmental Biology for a tenure-track appointment to start fall 2006. A Ph.D. and a strong commitment to excellence in liberal arts education are required. The successful candidate will teach a course in vertebrate developmental biology, courses in his/her specialty and team teach portions of the introductory biology course. The successful applicant will be expected to maintain an active research program with undergraduates. Illinois Wesleyan University is a nationally selective undergraduate liberal arts institution, located approximately 130 miles southwest of Chicago, with an enrollment of 2,100 students. Send curriculum vitae, undergraduate and graduate transcripts, statement of teaching philosophy, statement of research interests and how students might be involved, and three letters of recommendation to: **R. Given Harper, Chair, Department of Biology, Illinois Wesleyan University, P.O. Box 2900, Bloomington, IL 61702. E-mail: gharper@iwu.edu.** Review of applications will begin on 1 January 2006, and continue until the position is filled.

The Department of Statistics at North Carolina State University invites applications for a senior position **(ASSOCIATE/FULL PROFESSOR)** for its statistical genetics and bioinformatics programs. Responsibilities include teaching and research. All applicants must have a Ph.D. in statistics, or a related field, as well as a demonstrated interest and commitment for research at the intersection of statistical and biological sciences with an established record of funded research, collaboration, and good teaching. Send application or nomination letters, vitae, and names of three references to **e-mail: bioinformatics_search@stat.ncsu.edu** or to **fax: 919-515-1169.** For more information, see **website: http://www.stat.ncsu.edu.** *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

DIRECTOR
Division of Graduate Education
National Science Foundation,
Arlington, Virginia

National Science Foundation's (NSF) Directorate for Education and Human Resources seeks candidates for the position of Director, Division of Graduate Education (DGE). The Division leads the National Science Foundation's efforts to attract the most talented United States students into graduate studies, and to support them in their quest to become the leading scientists and engineers of the future. Information about the Division's activities may be found at **website: http://www.nsf.gov/ehr/dge/about.jsp.**

Appointment to this Senior Executive Service position may be on a career basis, on a one-to-three year limited term basis, or by assignment under the Intergovernmental Personnel Act (IPA) provisions.

Announcement S20060003A1, with position requirements and application procedures are posted on NSF's home page at **website: http://www.nsf.gov/about/career_opps/.**

Applicants may also obtain the announcements by contacting executive personnel staff at telephone: **703-292-8755** (Hearing impaired individuals may call **TDD 703-292-8044**). Applications must be received by January 15, 2006. *NSF is an Equal Opportunity Employer.*

EVOLUTIONARY DEVELOPMENTAL BIOLOGY. The Department of Biology at the University of Central Florida invites applicants for a tenure-track faculty appointment in evolutionary developmental biology at the rank of Assistant or Associate Professor. Candidates should have a strong focus on the mechanisms of phenotypic evolution in a broadly defined sense. The successful candidate will be expected to establish and maintain an extramurally-funded research program that complements our active and expanding faculty. The new faculty member will have the opportunity to participate in Ph.D. programs in biomolecular sciences and conservation biology, and contribute to graduate and undergraduate education. The University of Central Florida maintains a strong research emphasis with competitive startup funds and teaching loads. Candidates must have a Ph.D. and appropriate postdoctoral training. Please submit curriculum vitae, brief statements of research plans and teaching philosophy, and arrange for three letters of recommendation to be sent directly to: **Dr. Laurie von Kalm, Chair, Evolutionary Developmental Biology Search Committee, Department of Biology, University of Central Florida, 4000 Central Florida Boulevard, Orlando, FL 32816-2368.** Review of applications will begin January 15, 2006, with an anticipated start date of August 2006. See **website: http://www.cas.ucf.edu/biology/** for departmental details. Search documents may be viewed by the public upon request in accordance with Florida statute. *The University of Central Florida is an Affirmative Action/Equal Opportunity Employer.*

STAFF ASSOCIATE

The Staff Associate will be responsible for studies in the laboratory related to the kidney in injury and activation of regulatory molecules and pathways that underlie the injury phenotype. At least three years of experience performing all related molecular analyses on kidney tissues and vascular tissues. Extensive expertise in performing molecular analyses such as real time PCR, functional assays of migration and proliferation, western blotting, and signaling experiments related to these studies is essential.

Please send curriculum vitae and names of three references to: **Ms. Karen Evans, College of Physicians and Surgeons of Columbia University, P&S 17-401, 630 West 168th Street, New York, NY 10032.** *Columbia University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ASSISTANT PROFESSOR
Department of Biological Sciences
Auburn University

Assistant Professor, **GENOMIC PHYSIOLOGIST**. The Biological Sciences Department of Auburn University invites applications for a tenure-track position in genomic physiology. Candidates will be expected to possess expertise in genomic/operomic approaches to the study of integrated systems biology as they are used to understand the genomic responses that confer functionality and adaptation in plants or animals. Researchers taking a comparative or evolutionary approach to behavioral, environmental, or developmental responses are encouraged to apply. Duties include teaching in the Department's existing programs in physiology at the undergraduate and graduate levels, including a course in the candidate's area of specialization, and developing a vigorous, extramurally funded research program. Candidates whose research is complementary to a newly established Center for Environmental and Cellular Signal Transduction are encouraged to apply.

A Ph.D. and minimum of two years of postdoctoral research are required. *The candidate selected for this position, which will begin in August 2006, must meet eligibility requirements to work in the United States on date appointment is scheduled to begin and must be able to communicate effectively in English.*

Minorities and women are encouraged to apply. Applicants should submit curriculum vitae, statements of research interests and teaching philosophy, three representative reprints, and three letters of reference to: **Genomic Physiologist Search Committee Chair, Department of Biological Sciences, 101 Life Science Building, Auburn University, Auburn, AL 36849.** Review of applications will begin on January 15, 2006, and continue until the position is filled.

For more detailed information see **websites: http://www.auburn.edu/academic/science_math/biology/** or **http://www.auburn.edu/cmb.**

Auburn University is an Affirmative Action/Equal Opportunity Employer.

FACULTY POSITION IN NEUROSCIENCE
New Princeton Institute

Princeton University is seeking to make the first of several anticipated new faculty appointments in neuroscience, as part of its new Institute in this area and its growing focus on quantitative approaches to understanding neural coding and dynamics. The position is at the Assistant Professor level, to begin in September 2006, for a Theorist in systems and/or cognitive neuroscience. The appointment will be joint between the Institute and a department appropriate to the individual's background and interests, with possibilities including (but not limited to) psychology, molecular biology, mathematics, physics, electrical engineering or computer science. Applicants should be prepared to teach both an undergraduate and a graduate level course in neuroscience. Please send curriculum vitae, a one-page research description, and three letters of recommendation to: **Search Committee, Neuroscience Institute, Princeton University, Princeton, NJ 08544,** or **e-mail: search@neuroscience.princeton.edu.** Materials should be submitted as soon as possible. Applications will be considered on a rolling basis, and the search will remain open until the position is filled. For information about applying to Princeton and how to self-identify, please link to **website: http://web.princeton.edu/sites/dof/ApplicantsInfo.htm.** *Princeton is an Equal Opportunity, Affirmative Action Employer.*

POSTDOCTORAL ASSOCIATE to conduct research on multiple myeloma. The applicant should have Ph.D., and be motivated, organized, and able to work independently. Knowledge of cell culture, basic molecular biology, and computer skills are required. The applicant must have United States work experience. Salary range is $36,000 to $42,000. Submit curriculum vitae and two letters of recommendation to: **Dr. O. Batuman, State University of New York (SUNY) Downstate Medical Center, Box 20, Brooklyn, NY 11203** or **e-mail: obatuman@downstate.edu.**

www.admin.cam.ac.uk/jobs/

The Professorship of Physical Chemistry

The Board of Electors to the Professorship of Physical Chemistry invite applications for this Professorship, which has fallen vacant on the retirement of Professor Sir David King.

Applications are sought from individuals whose expertise falls within any area of physical chemistry, and who have an internationally leading track record of research.

Appointment will be from 1 October 2006 or as soon as possible thereafter.

Further information may be obtained from the Academic Secretary, University Offices, The Old Schools, Cambridge CB2 1TT. E-mail: ibise@admin.cam.ac.uk Applications should be sent, together with details of current and future research plans, a curriculum vitae, a publications list, contact details for three professional referees and form PD18 (downloadable from http://www.admin.cam.ac.uk/offices/personnel/forms/pd18), so as to reach him no later than 31 January 2006.

Informal enquiries may be made to Professor Jeremy Sanders, Head of the Department of Chemistry E-mail: jkms@cam.ac.uk

UNIVERSITY OF CAMBRIDGE

The University offers a range of benefits including attractive pension schemes, professional development, family friendly policies, health and welfare provision, and staff discounts. The University is committed to equality of opportunity.

CHALMERS

Two Faculty Positions in Quantitative Systems Biology

Chalmers Biocenter is a strategic initiative at Chalmers to jumpstart new fields in bioscience and strengthen existing cutting edge research in the area. We are currently looking to fill two positions in Quantitative Systems Biology, with emphasis on experimental and computational approaches, respectively. Of particular interest are individuals who combine modeling and experiments to understand and exploit properties of biological systems. The positions are at the full professor level, but consideration will also be given to more junior applicants.

Researchers from all backgrounds relevant to Quantitative Systems Biology are encouraged to apply, including but not limited to bioimaging, image analysis, modeling, chemical biology, cell and molecular biology, or systems analysis of biological networks.

Please read more on our website: **www.chalmers.se/biocenter** and under Vacancies on **www.chalmers.se/en/**

Further information can also be obtained from the coordinator of the Chalmers Biocenter, Catharina Hiort + 46 31 772 86 33.

Deadline for receipt of completed applications is **February 17, 2006**.

CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden

COLUMBIA UNIVERSITY

Motor Neuron Center Faculty Recruitment

Columbia University Center for Motor Neuron Biology and Disease is recruiting faculty with interests in motor neuron biology, ALS or SMA to join a translational program involving basic and clinical research. We are particularly keen to attract individuals using molecular, genetic, chemical, cellular, electrophysiological and/or imaging approaches to the following areas: cellular, axonal and synaptic aspects of neurodegeneration; CNS gene therapy; motor control circuits; functional imaging of the spinal cord; genetics of ALS; SMN biology; preclinical testing of therapeutic strategies; and locomotion. We encourage applications for positions at the Assistant Professor level, but will also consider applications from more senior investigators for positions at the level of Associate or full Professor.

Columbia University currently has a world-renowned program in neurobiology and behavior and in medical and surgical neurology. The new Motor Neuron Center aims to enhance interactions between basic and clinical researchers in the field; Center members will have access to core facilities including high-throughput screening. Faculty will have opportunities for strong ties and academic appointments with the Center for Neurobiology and Behavior, scientific departments and programs on Columbia campuses.

Applications for this round of recruitment are requested by January 20, 2006. A C.V., cover letter including statement of interests, and three letters of reference under separate cover should be e-mailed care of **Dr. Serge Przedborski, sp30@columbia.edu.** In addition, please mail a hard copy of these documents to:

Motor Neuron Center Search Committee
c/o: Dr. Serge Przedborski
Columbia University Medical Center
William Black Building
Room 302
650 West 168th Street
New York NY 10032

Columbia University takes affirmative action to ensure equal employment opportunity.

POSITIONS OPEN

The Raymond F. Baker Center for Plant Breeding, the Department of Agronomy, and the Plant Sciences Institute seek to fill an **ENDOWED PROFESSORSHIP** in plant breeding and genomics, focused on developing crops as biorenewable resources using molecular and conventional approaches. The position will be part of a cluster of scientists focused on using plant biomass for biorenewable products and energy. The successful candidate will be expected to develop a vigorous, extramurally funded research program investigating plant composition and to produce germplasm with improved yield and quality. Research by the incumbent should further goals of crop diversification to support rural economic development and improved environmental stewardship. Possible crops include, but are not limited to, maize, sorghum, and switchgrass. Teaching and advising graduate and undergraduate students and participation in the development of plant breeding and genetics curricula is expected. Required qualifications: Ph.D. in plant breeding, plant molecular biology, genetics, or related field, strong statistical skills, demonstrated evidence of research and teaching proficiency, excellent written and oral communication skills, and experience working collaboratively with researchers, processors, and consumers. Preferred qualifications: A background in plant breeding field methods and laboratory analyses, plant pathology, entomology, and agronomic crop production. Ability to work with farmers and industry partners. Experience with industrial agricultural products desirable. Salary: Commensurate with qualifications.

Application instructions: Applicants should submit a complete resume including e-mail address, graduate transcripts, and names and contact information of three references to: **Dr. Steven Fales, Chair, Department of Agronomy, 2101 Agronomy Hall, Iowa State University, Ames, IA 50011.**

FACULTY POSITION
Microbiology

The Department of Microbiology and Immunology at Des Moines University, Osteopathic Medical Center invites applicants for a tenure-track position at the level of Assistant or Associate Professor. The successful candidate must have a demonstrated expertise in teaching microbiology, particularly in the area of pathogenic bacteriology. In addition, it is expected that the individual develop an innovative and extramurally funded research program using contemporary approaches to study host-pathogen interaction. Applicants should have a Ph.D. and relevant postdoctoral experience. Rank and salary are commensurate with training and experience. Applicants should send curriculum vitae, a concise statement of teaching and research interests, and the names of three professional references to:

Microbiology Faculty Search
Department of Microbiology and Immunology
Des Moines University
Osteopathic Medical Center
3200 Grand Avenue
Des Moines, IA, 50312

Visit **website: http://www.dmu.edu.** For full consideration applications should be received by January 30, 2006.

FACULTY POSITIONS
The University of Southern California Norris Comprehensive Cancer Center

The University of Southern California (USC)/Norris Comprehensive Cancer Center of the Keck School of Medicine at the University of Southern California seeks applicants for faculty positions at the Assistant, Associate, and Full Professor level in the following areas of cancer research: apoptosis, autophagy, cancer stem cells, DNA repair and stress response which contributes to cancer progression and drug resistance. Each applicant should send current curriculum vitae, research plan, and three letters of reference to: **Dr. Amy Lee, University of Southern California/Norris Comprehensive Cancer Center, 1441 Eastlake Avenue, MC-9181, Los Angeles, CA 90089-9181.** *USC is an Equal Opportunity Employer.*

The Agronomy Department at Iowa State University (ISU) seeks to fill a tenure-track **ENDOWED PROFESSORSHIP** in crop genomics to apply genomic data and related technological resources toward the development of sustainable cropping systems. The successful candidate will provide leadership and vision in the translation of genomic, functional genomics, and computational technologies to crop improvement, and must demonstrate a clear understanding of these modern technologies and an ability to integrate them to link genotype and phenotype. Possible research areas for the application of modern genomics tools include, but are not limited to, developing superior breeding theory, understanding genetics and physiology of crop performance, or developing gene-based sustainable cropping systems. The successful candidate will conduct integrative interdisciplinary research with plant molecular biologists, bioinformaticists, physiologists, and plant breeders on crop(s) and biorenewable resources of agronomic importance to Iowa and the Midwest. Teaching and advising graduate and undergraduate students is expected. Exceptional opportunities for collaborative research exist with ISU faculty and USDA-Agricultural Research Service (ARS) scientists on the ISU campus who are active in world-class programs for crop improvement, bioinformatics and computational biology. This Endowed Professorship complements a substantial commitment of resources by USDA-ARS for plant genome database development and management on the ISU campus. Required qualifications: Ph.D. in plant genomics, plant breeding, plant molecular biology, quantitative/population genetics, crop physiology/ecology, plant bioinformatics, or related field; and demonstrated evidence of research and teaching proficiency. The incumbent must have experience in collaborative and interdisciplinary research and a proven capacity to integrate information across levels of biology complexity. Preferred qualifications: Experience using genome databases and an understanding of whole plant physiology and plant breeding. An excellent record of extramural funding, mentoring graduate students, and teaching. Salary: Commensurate with qualifications. Application instructions: Applicants should submit a complete resume including e-mail address, graduate transcripts, and names and contact information of three references to: **Dr. Steven Fales, Chair, Department of Agronomy, 2101 Agronomy Hall, Iowa State University, Ames, IA 50011.**

The Radiation Oncology Department of the Medical College of Wisconsin (MCW) in Milwaukee seeks a **POSTDOCTORAL FELLOW** for the NIH-funded MCW Center for Medical Countermeasures against Radiological Terrorism. This position will enhance and develop the study of experimental radiation nephropathy, particularly the role of chronic oxidative stress. There will be collaboration with other members of the Center, in drug development, radiation physics, and normal tissue radiobiology of lung, brain, and gastrointestinal tract. Experience in molecular biology, biochemistry, and/or physiology is desirable. Experience in radiobiology is preferred, not required. Experience in writing for publication is required, as is a passing grade on the Test of English as a Foreign Language (TOEFL). This is a two year appointment contingent on performance, with possibility of renewal. Contact **e-mail: yvonnem@mcw.edu** for more information. *MCW is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION available January 1, 2006, to study Helicobacter pylori induction of ulcers/cancer in animals using antibody and microarrays and realtime PCR. The Department features outstanding core facilities and faculty (**website: http://www.sh.lsuhsc.edu/microbiology**). Send curriculum vitae, two letters of reference, and one page statement of career aspirations to: **Dr. David J. McGee, e-mail: dmcgee@lsuhsc.edu.** *Louisiana State University is an Equal Opportunity/Affirmative Action Employer.*

GASTROINTESTINAL ONCOLOGIST
The University of Washington and The Fred Hutchinson Cancer Research Center

The University of Washington School of Medicine and the Fred Hutchinson Cancer Research Center (FHCRC) seek a full-time oncology faculty member in the Clinician/Teacher pathway with an interest in gastrointestinal or pancreatic malignancies. The primary appointment will be at the Assistant or Associate Professor level with a joint appointment at the FHCRC, if desired. The selected candidate should be a M.D. with experience in clinical research plus a major commitment to patient care and teaching.

The new clinical infrastructure, the Seattle Cancer Care Alliance, is a dynamic, rapidly growing, well-supported collaboration between the University of Washington, Fred Hutchinson Cancer Research Center and Children's Hospital and Regional Medical Center.

Candidates should submit curriculum vitae, at least four names for references, and a concise statement of career goals, which should identify the candidate's area of clinical research. A letter of application should be addressed to: **Gastrointestinal Oncology Search Committee, Attn: Dan Meenach, Faculty Coordinator, FHCRC, 1100 Fairview Avenue N., Mailstop D5-310, Seattle, WA 98109.** The closing date for application is January 16, 2006.

The University of Washington and the Fred Hutchinson Cancer Research Center are Affirmative Action, Equal Opportunity Employers. Both institutions are dedicated to the goal of building a culturally diverse and pluralistic faculty and staff committed to teaching and working in a multicultural environment and strongly encourage applications from women, minorities, individuals with disabilities and covered veterans.

ASSISTANT PROFESSOR OF GENETICS

The University of Nebraska, Omaha announces a tenure-track Assistant Professor position starting August 2006. The position requires an earned Ph.D. and postdoctoral experience. The successful candidate will contribute to an established degree program in biotechnology through teaching, research, and student advising. We seek a candidate that will teach courses in the area of genetics and molecular genetics and establish a vigorous research program. For more information see **website: http://www.unomaha.edu/biology/.** Screening of applications will begin January 18, 2006, and continue until the position is filled. Applications must be submitted through the University human resources **website: http://careers.unomaha.edu** and should include curriculum vitae and statements of teaching and research objectives. Have three letters of recommendation sent to: **William Tapprich, Chair, Department of Biology, University of Nebraska, Omaha, 6001 Dodge Street, Omaha, NE 68182-0040.** *The University and Department are strongly committed to achieving diversity among faculty. We are particularly interested in receiving applications from members of underrepresented groups and encourage women and persons of color to apply.*

POSTDOCTORAL POSITION
Howard Hughes Medical Institute Supported

Canisius College has a two-year Interdisciplinary Science Teacher-Scholar Postdoctoral Fellowship available for fall 2006. The successful applicant will join a team of faculty teaching introductory biology, and one upper elective course in his/her area of specialization during his/her second year. He/she will join an on-going interdisciplinary or collaborative research team or continue his/her own work (a small research budget is included) and involve undergraduates. Additional information may be found at **websites: http://www2.canisius.edu/~dehn/** (this position), **www.canisius.edu/biology** (the Department) or by contacting **e-mail: dehn@canisius.edu.** Send a letter of application, current curriculum vitae, a statement of teaching philosophy and research interests, transcripts, and contact information for references to: **Dr. Paula Dehn, Professor and Chair Biology, 2001 Main Street, Buffalo, NY 14208** by January 30, 2006.

POSITIONS OPEN

EVOLUTIONARY GENETICS/GENOMICS
Harvard University
Department of Organismic and Evolutionary Biology

The Department of Organismic and Evolutionary Biology at Harvard University seeks to make an appointment at the junior rank in the field of evolutionary genetics/genomics. We seek an outstanding scientist who will establish an empirical research program and teach both undergraduate and graduate students. The candidate would have the opportunity to interact with faculty from other departments in the faculty for arts and sciences as well as at the Broad Institute/MIT and Harvard Medical School. We are especially interested in individuals who conduct rigorous, field and/or laboratory-based tests of general problems associated with the genetic basis of adaptations in natural populations. We encourage applications from or information about women and minority candidates.

Applicants should submit curriculum vitae, statements of research and teaching interests and representative publications, and should arrange for three letters of reference to be sent to: **Professor John Wakeley, 2102 Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA, 02138.** Nominations from third parties are also welcome. Review of applications and nominations will begin February 1, 2006.

Further information about the Department is available at its **website: <http://www.oeb.harvard.edu>.**

Harvard University is an Affirmative Action/Equal Opportunity Employer.

The University of Florida McKnight Brain Institute (MBI), Shands Cancer Center (UFSCC), the Department of Neurological Surgery, and the Department of Pharmacology and Therapeutics in the College of Medicine are expanding our Neuro-Oncology program with a focus on molecular therapeutics. Positions are open for tenure-track **NEURO-ONCOLOGY RESEARCHERS** having a Ph.D. and/or M.D. degree, at the Assistant/Associate/Full Professor levels. We are especially interested in the fields of cancer stem cell biology, immunology, and cell signaling with an eye toward the development of new cellular and molecular therapeutic approaches for brain cancer. Substantial startup packages and endowments will support the investigators to help build a program dedicated to the development of new therapeutics for invasive brain tumors. Additional information about the research and educational programs in the MBI, UFSCC and the Department are available at **websites: http://www.mbi.ufl.edu, www.ufscc.ufl.edu** and **www.med.ufl.edu/pharm,** respectively. Applications consisting of curriculum vitae and three letters of recommendation will be accepted until December 31, 2005. Please contact: **Dr. Dennis A. Steindler, Executive Director, The Evelyn F. and William L. McKnight Brain Institute of the University of Florida, College of Medicine, P.O. Box 100015, Gainesville, FL, 32610. E-mail Steindler@mbi.ufl.edu.** *This is an Equal Opportunity Institute.*

PROFESSOR AND HEAD, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station. A description of the Department and a complete position description are available at **website: http://vtpb-www.cvm.tamu.edu.** Candidates must have an earned doctoral degree in a relevant scientific field and have demonstrated understanding of veterinary medicine. Applications should include curriculum vitae and a letter describing the candidate's qualifications and administrative philosophy, along with the names, addresses, telephone numbers, and e-mail addresses of four references. Send applications electronically to: **Dr. Deborah Kochevar, Chair, Search Committee** at **e-mail: dkochevar@cvm.tamu.edu.** Application review will begin February 15, 2006, and will continue until the position is filled. For questions, e-mail aforementioned address, or call **telephone: 979-845-3878.** *Affirmative Action/Equal Employment Opportunity Employer.*

University of Nebraska Medical Center

POSTDOCTORAL POSITION IN STAPHYLOCOCCUS AUREUS

A Postdoctoral Position is available immediately to study the molecular mechanisms of antibiotic-induced killing in Staphylococcus aureus. A Ph.D. and experience with molecular/biochemical techniques is required. The salary is commensurate with experience and funding is available for three years. Send curriculum vitae and three letters of recommendation to: **Kenneth W. Bayles, Department of Pathology and Microbiology, University of Nebraska Medical Center, 986495 Nebraska Medical Center, Omaha, NE 68198-6495.** Review of applications will begin January 1, 2006, and will continue until qualified applicants have been identified. More information on Dr. Bayles' research can be found at **website: http://www.unmc.edu/Pathology/facultypages/baylesbio.htm.** *University of Nebraska Medical Center is an Equal Opportunity/Affirmative Action Employer. Minorities and women are encouraged to apply.*

ASSISTANT PROFESSOR of biology, University of South Carolina, Sumter, tenure-track, begins fall 2006. Ph.D. in biology. Twelve hours per semester, all undergraduate, expectations include excellence in teaching and potential for research/scholarship. Ability to teach introductory biology courses, ecology/evolution, and environmental science with accompanying field work to majors and nonmajors. May apply online at **website: http://uscjobs.sc.edu** or submit application letter (should describe the applicant's record, philosophy of teaching, and professional goals and interests), curriculum vitae, three current letters of recommendation, copies of all undergraduate and graduate transcripts, writing samples, and summary of teaching evaluations, or other evidence of excellence in teaching. Send materials to: **Charles F. Denny, Division of Science, Math and Engineering, University of South Carolina, Sumter, 200 Miller Road, Sumter, SC 29150-2498.** Review of credentials will begin immediately. *Foreign nationals indicate current United States immigration status. Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION. This laboratory is interested in hiring a Postdoctoral Fellow with a deep interest in all levels of protein function: structure, dynamics, ground and transition-state structure and energetics, ligand-binding, allostery, the conformational coupling of energetics, and the higher-order organization of catalysis in the cell. Our projects, many of which are structurally grounded, include numerous enzymes that are loosely centered around biomedically relevant issues in sulfur metabolism, isoprenoid biosynthesis and antibiotic development. Please send or e-mail your resume and three letters of recommendation to: **Professor T.S. Leyh, Department of Biochemistry, The Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: leyh@aecom.yu.edu.** *Equal Opportunity Employer.*

ASSISTANT/ASSOCIATE PROFESSOR BIOFILM MICROBIOLOGIST

Montana State University (MSU) seeks a Biofilm Microbiologist for a tenure-track position that will be a joint appointment in the Department of Microbiology and Center for Biofilm Engineering (CBE). Responsibilities include teaching undergraduate and graduate courses, developing a funded research program, and contributing to the interdisplinary education, industrial interaction, and collaborative research programs of the CBE. For more information and to apply please see our **website: http//www.montana.edu/level2/jobs.html.** Screening of applications begins January 16, 2006, and will continue until the position is filled. *MSU-Bozeman is an ADA/Affirmative Action/Equal Opportunity/Vetarans' Preference Employer.*

ASSOCIATE PROFESSOR/ASSISTANT PROFESSOR. The Department of Internal Medicine, Rheumatic Diseases Division at University of Texas Southwestern Medical Center, Dallas is seeking a Ph.D., M.D. or M.D./Ph.D. with a minimum of five years of postgraduate experience to develop and maintain an independent basic and/or translational research program in autoimmune diseases including but not limited to systemic lupus erythematosus and rheumatoid arthritis. Candidates should have a demonstrated record of scientific productivity in the form of publications and extramural funding. Current NIH funding is highly desirable. This position is intended to provide current division members with an additional opportunity for collaboration and program development. Training and supervision of graduate and postgraduate trainees will be required. The candidate's credentials and experience will determine academic rank. Send letter of interest (including description of research area and future plans, curriculum vitae and names of three or more references to:

David R. Karp, M.D, Ph.D.
Associate Professor and Chief
Rheumatic Diseases Division
University of Texas Southwestern Medical Center
5323 Harry Hines Boulevard
Dallas, TX 75390-8884
E-mail: David.Karp@utsouthwestern.edu

FACULTY POSITION
Molecular Biophysics
Johns Hopkins University
School of Medicine

The Department of Biophysics and Biophysical Chemistry (**website: http://biophysics.med.jhmi.edu**) seeks outstanding candidates for the position of Assistant Professor. Applications are sought in all areas of molecular biophysics and biophysical chemistry, including structural biology. Priority will be given to applications received by February 1, 2006. Please submit curriculum vitae, a summary of current and proposed research, and arrange to have three letters of recommendation sent to:

Search Committee
Department of Biophysics and Biophysical Chemistry
Johns Hopkins University
School of Medicine
WBSB 713
725 North Wolfe Street
Baltimore, MD 21205-2185
Fax: 410-502-6910
E-mail: biophysjob@bs.jhmi.edu

The Johns Hopkins University is an Equal Opportunity Employer.

The Department of Biology (**website: http://www.usd.edu/biol/**) invites applications for an **ASSISTANT PROFESSOR** (nine-month, tenure-track) in ecology. We seek an individual with a broad background in ecology, interested in integrative and collaborative research, who will develop a creative externally funded research program, and exhibit excellence in teaching/mentoring of undergraduate and graduate students. Candidates whose research explores questions above the population level, or with interests in the ecology of wetlands, riparian zones, or aquatic systems are especially encouraged. Ph.D. required; postdoctoral, research/teaching experience preferred. Salary commensurate with rank and qualifications. Send a letter of application, curriculum vitae, statement of research/teaching interests, and contact information for three references to: **Ecology Search, Department of Biology, The University of South Dakota, 414 East Clark Street, Vermillion, SD 57069.** Questions should be directed to: **Dr. D.A. Soluk (E-mail: dsoluk@usd.edu).** Review of applications begins January 14, 2006, and continues until the position is filled. *The University of South Dakota is an Equal Opportunity/Affirmative Action Employer committed to increasing the diversity of its faculty, staff and students.*

COURSE

Drew University
Residential School on Medicinal Chemistry: Chemistry and Biology in Drug Discovery
Madison, New Jersey – June 12-16, 2006

The Residential School is a week-long graduate level course organized to provide an accelerated program for medicinal chemists and biologists who wish to broaden their knowledge of small molecule drug discovery and preclinical development. The course is focused on fundamental concepts that are useful in drug discovery spanning initial target validation, enzyme and receptor assays, high throughput screening, hit-to-lead progression, lead profiling and modification, structure-based drug design, QSAR, plasma protein binding, pharmacokinetics, metabolism, drug delivery, toxicology and patents. Case histories of recent successful drug discovery and development programs will also be presented. Attendance is limited to 200 participants with preference given to applicants having five years or less industrial experience.

A Special Topics Course on **Designing Drugs with Optimal *In Vivo* Activity After Oral Administration** is also being offered on **June 19-20**. This course, which is limited to 40 participants, will cover topics relevant to the design of orally active drugs including GI physiology, oral bioavailability, formulation, prodrug strategies, pharmacokinetics and pharmacodynamics, intestinal and hepatic metabolism, biliary and renal clearance, and appropriate case histories. Previous attendance at the Residential School on Medicinal Chemistry is not a prerequisite for acceptance to the Special Topics Course.

More information and application forms can be obtained at www.depts.drew.edu/resmed or by contacting the Residential School's Office at Drew University, Hall of Sciences, Room 319, Madison, NJ 07949, USA; Phone: 973/408-3787, Fax: 973/408-3504, E-mail: resmed@drew.edu

CONFERENCE

Genomes to Systems Conference 2006

22-24 March 2006
Manchester, UK

Genomes to Systems is back with a **3-day programme** of **16 academic sessions** from over **50 world-class speakers**, covering the full spectrum of **post-genomic disciplines.**

Plenary speakers by:

Bernard Palsson (San Diego)
Denis Noble (Oxford)
Julie Ahringer (Cambridge)
Matthias Uhlen (Stockholm)
Michael Snyder (Yale)

The Conference includes an Exhibition, Poster Sessions, Network Evening and Technology Showcases.

Early-bird and Group discounts available

For the full programme and registration visit:
www.genomestosystems.org

The Consortium for Post-Genome Science

Gene Expression & Signaling in the Immune System
April 26 - 30, 2006

Organized by:
Doreen Cantrell, *University of Dundee*
Richard Flavell, *HHMI/Yale University*
Rudolf Grosschedl, *University of Munich*
Stephen Smale, *HHMI/UCLA Sch. of Med.*

Topics include:

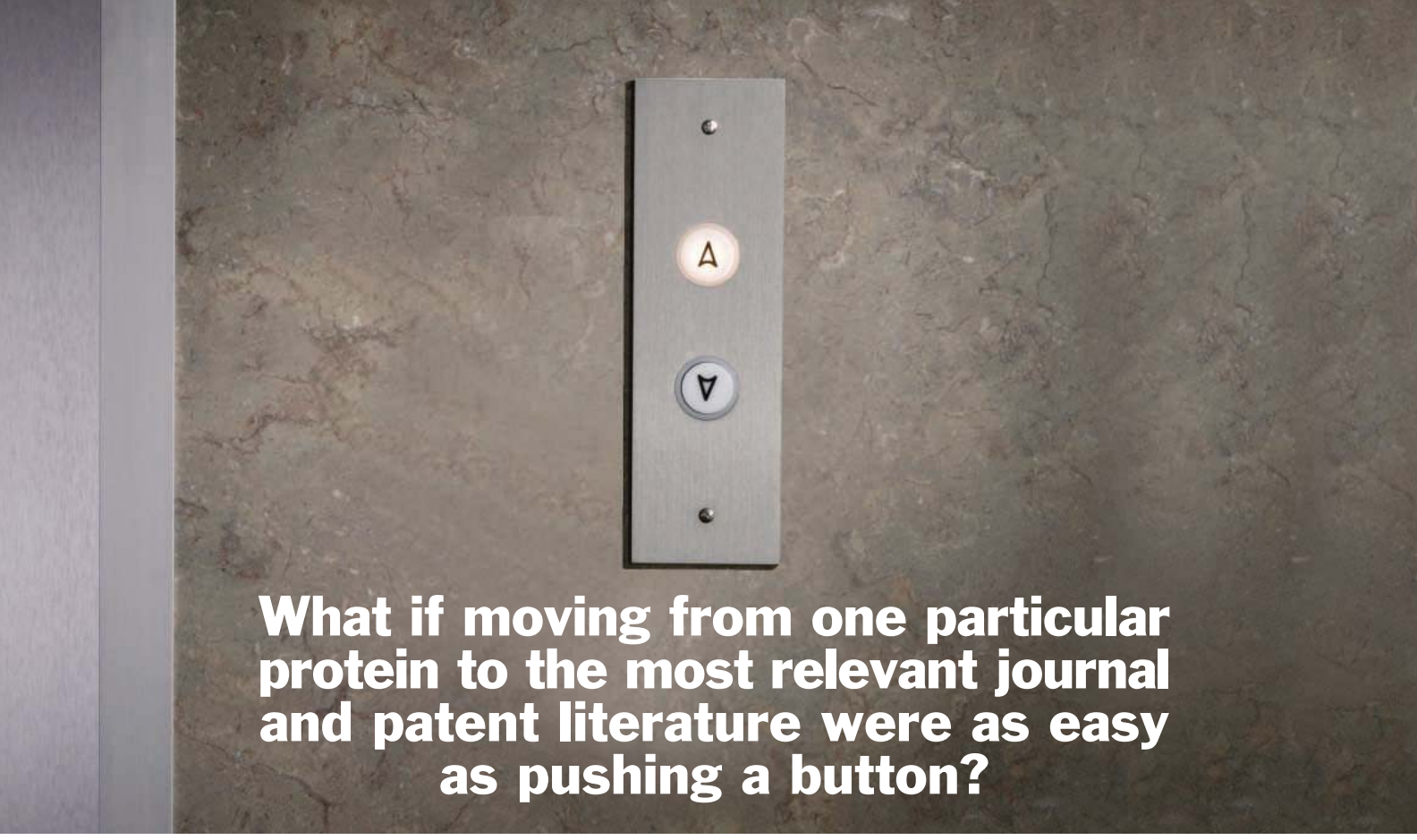
· Stem Cells/Early Developmental Decisions
· Regulation of Lymphocyte Development
· Control of Antigen Receptor Gene Assembly
· Antigen Receptor Signaling
· Cell Death · Innate Immunity
· Cytokine Signaling · Cell Activation

Speakers include: Shizuo Akira, Frederick Alt, David Baltimore, Yehudit Bergman, Meinrad Busslinger, Kathryn Calame, Gerald Crabtree, Mark Davis, Sankar Ghosh, Laurie Glimcher, Gillian Griffiths, Cynthia Guidos, Douglas Hilton, Dimitris Kioussis, Gary Koretzky, Michael Krangel, Dan Littman, Tak Mak, Diane Mathis, Ruslan Medzhitov, Matthias Merkenschlager, Kenneth Murphy, Cornelis Murre, Michael Neuberger, Michel Nussenzweig, Klaus Rajewsky, Anjana Rao, Steven Reiner, Tannishtha Reya, Alexander Rudensky, David Schatz, Mark Schlissel, Andrey Shaw, Harinder Singh, Tadatsugu Taniguchi, Alexander Tarakhovsky, Craig Thompson, Jurg Tschopp, Ulrich von Andrian

Abstract Deadline: February 1, 2006

Cold Spring Harbor Laboratory
PO Box 100, Cold Spring Harbor NY 11743
Tel 516 367 8346 Fax 516 367 8845
meetings@cshl.edu www.cshl.edu/meetings

MARKETPLACE



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Visualization tools help you understand results at a glance. You can categorize topics and substances, identify relationships between areas of study, and see areas that haven't been explored at all.

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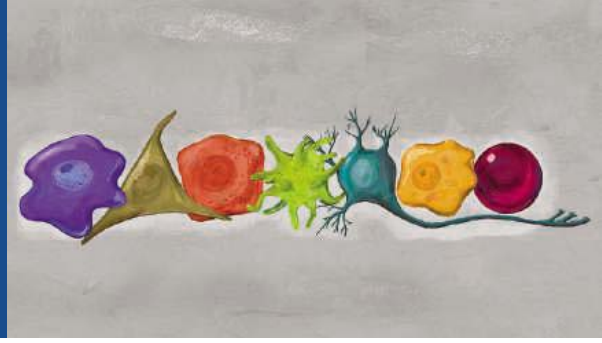
Neural Stem Cells | from R&D Systems



Quality | Selection | Performance | Results

High Nestin Expression:

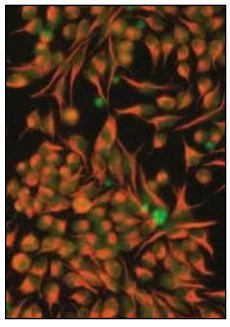


FIGURE 1. High level of Nestin expression detected in R&D Systems' rat cortical stem cells (Catalog # NSC001) using goat anti-rat Nestin polyclonal antibody (Catalog # AF2736). Cells were stained using Rhodamine Red-conjugated anti-goat secondary antibody and counterstained using Fluoro Nissl Green.

Monolayer/Neurosphere Expansion:

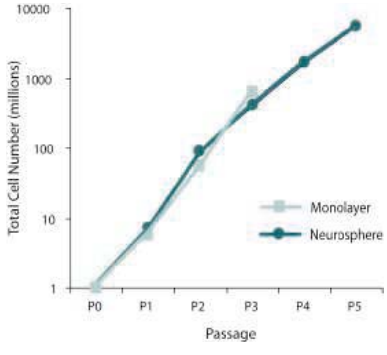
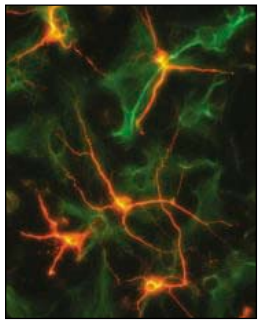
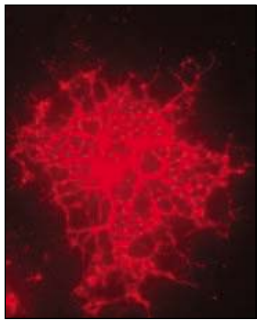


FIGURE 2. Expansion of rat cortical stem cells (Catalog # NSC001). For monolayer culture, cells were expanded using R&D Systems Fibronectin-coated plates (Catalog # 1030-FN) and StemXVivo Serum-Free NSC Base Media (Catalog # CCM002) supplemented with recombinant human FGF basic (Catalog # 233-FB; 20 ng/mL). For neurosphere culture, cells were expanded using the StemXVivo Serum-Free NSC Base Media supplemented with recombinant human FGF basic and EGF (Catalog # 236-EG; 20 ng/mL).

Multipotency:



(A)



(B)

FIGURE 3. Detection of cell populations expressing neuron-specific β-III tubulin (Catalog # MA1195, Red) and glial fibrillary acidic protein (Catalog # AF2594, Green) (A) and oligodendrocyte marker O4 (Catalog # MAB1326) (B) in differentiated rat cortical stem cells.

Ex vivo expanded neural stem cells serve as excellent tools for researchers studying neural development and neurological disorders. R&D Systems now offers ready-to-use primary cortical stem cells (Catalog # NSC001) isolated from E14.5 Sprague-Dawley rats.

Features:

- **Quality & Consistency**
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  - Ready-to-use
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Germany
R&D Systems GmbH
Tel: 0800 909 4455
infogmbh@RnDSystems.co.uk

France
R&D Systems Europe
Tel: 0800 90 72 49
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